SOME ASPECTS OF DEVELOPMENTAL PHYSIOLOGY IN PEAS

being a thesis

presented by

JAMES I. SMITH

for the degree of

Doctor of Philosophy

in the

University of Tasmania

May 1958

A C K N O W L E D G E M E N T S

I wish to thank my supervisor, Professor H.N. Barber, for the help he has given during all stages of this work. I am indebted to Professor Barber and several other members of the research group of the Botany Department for making available unpublished data.

The statistical advice of my husband has been of great assistance in interpreting some of the data.

The willing assistance of the technical staff of the University of Tasmania is gratefully acknowledged.

This work has been carried out while I was in receipt of the I.C.I.A.N.Z. Research Fellowship of the University of Tasmania. My thanks are due to I.C.I. for making this Fellowship available to me.

C O N T E N T S

CHAPTER 1.

i General Introduction

Since the classic work of Mendel the garden pea, Pisum sativum L. has been used extensively for genetical and physiological investigations. During the last 20 years de Haan (1927), Lamm (1937) and Wellensiek (1925) have located many of the gene positions and described many interesting genotypes.

With regard to stature and flowering behaviour, commercial pea varieties can be divided into approximately four main groups which are summarized in the following table. The examples given are the varieties most commonly used in the experiments to be described in this thesis.

Description	Example	Genetic Constitution
Late Tall	Telephone	Sn Le
Late Dwarf	Greenfeast	Sn le
Early Tall	Alaska	sn Le
Early Dwarf	Massey	sn le

It can be seen from this table that the four groups represent all combinations of the genes Sn and sn with Le and le. These genes control the flowering behaviour and length of pea plants and segregate in a simple Mendelian manner. Sn governs the node at which the first flower is produced and is also one of the main determinants of the number of days to flower production. Plants possessing the dominant Sn are facultative long day plants and respond to vernalization, (Barber, 1958), whereas sn plants are day-neutral and show no vernalization response. Barber and Paton (1952, 1955) showed that Sn plants are able to produce a substance inhibitory to flowering. Barber (1958) has proposed the name "colysanthin" for this substance. Colysanthin is present in the cotyledons of late pea varieties and during the first two weeks after germination it passes into the plumule of the seedling (see Chapter 2).

Paton (1956) has suggested that in short days colysanthin can be produced by the leaves, which inactivate this substance in long days, probably by converting it into a flower hormone. The action of vernalination on late varieties is also to remove colysanthin, and the responses to vernalization and long days are competitive (Harter 1958).

Haupt (1952, 1955 a and b) proposed two other mechanisms by which flowering in peas may be controlled. The first is that a flower promoting substance is present in the cotyledons of some varieties (presumably early) of peas. This has been confirmed in part in this laboratory (see Chapter 2). However, it is difficult to separate this hypothesis from Haupt's second proposal that vegetative growth in itself inhibits flowering. This suggestion has been put forward to explain the behaviour of various plants (see Holdsworth 1956 for discussion) and undoubtedly can explain some experimental results. Haupt's early evidence in suggesting competition between flowering and reproductive growth includes data showing that yeast extract can delay flowering in the early variety "Kleine Rheinländerin" (cultured without cotyledons). More recently, Haupt (1957a) has been able to vary the node of first flower in this variety in "embryo" cultures by applying photoperiodic treatments. Thus in darkness and in long days the node of first flower (F) is approximately the same, but in short days it is significantly higher. When Haupt's results are compared with these obtained in this department, they seem most easily explained as the production of colysanthin in short days. The substrate for colysanthin production, not normally present in early varieties, was provided by Haupt in the culture medium. When such a substrate is provided, early and late pea varieties respond to photoperiod in a similar manner, as Haupt has shown using "Alderman" as his late variety. Thus it appears that plants with the recessive gene *en* are unable to carry out one step in the reaction chain leading to the production of colysanthin. This type of situation has been found frequently in biochemical genetics.

Although Sn is the major locus governing flowering node Barber (1958), as a result of crossing experiments, has postulated two minor systems. One of these is a modifying system and the other a separate system of polygenes. The latter is probably not sensitive to photoperiod.

The Sn locus shows a number of pleiotropic effects, only two of which (sensitivity to photoperiod and vernalization) have so far been mentioned. Normal varieties of pea produce two scale leaves, then a number of nodes with two leaflets followed by nodes with three or four leaflets. The node at which more than two leaflets are produced varies with Sn constitution of the plant. In addition the dominant Sn probably causes small reduction in stem length. These relationships were hinted at by Tedin and Tedin (1923) and described by Barber (1958).

While the discussion given above accounts for the flowering behaviour of most pea varieties, there are exceptions. The most interesting of these is a recessive "acacia" (tl tl) mutant whose Sn constitution is unknown. This genotype initiates flowers at about node six or seven, but the flowers abort at an early stage. Succeeding nodes produce flowers which develop slightly further until a functional flower is produced at about node nine, the actual figure varying with external environment. This "acacia" mutant has other points of interest. Its distinguishing feature is that it bears no tendrils. Normal pea varieties have a number of leaflets and a number of tendrils (which may be considered reduced leaflets). The factors governing the production of leaflets instead of tendrils are not known, but in the "acacia" mutant all possible sites are occupied by leaflets, to the exclusion of tendrils. Kujala (1953) found a mutant with no leaflets and all sites occupied by tendrils. Unfortunately this has been lost and has so far not reappeared, but it would be very useful for physiological comparison with "acacia".

The genetics of leaflet production is less well known than that for flowering. The acacia mutant bears the recessive gene tl and most normal varieties the dominant Tl. The maximum number of leaflets produced at a node varies with variety. The extreme case is the "acacia" mutant which has up to about 14 leaflets in one leaf.

Massey rarely produces more than four leaflets but Greenfeast and Telephone regularly produce six leaflets at about nodes 16-20. This may be a secondary effect of Sn, since Massey matures early, before sufficient vegetative growth has taken place for six-leaflet leaves to be produced. Numerous genes are known which effect leaf shape and colour (glaucous or green) in peas. Very little is known about the physiology of the different leaf forms. Work on leaf development to be described in this thesis is largely confined to factors affecting leaflet number.

In normally growing plants leaflet number is an adequate estimate of leaf area (see chapter 2) as well as being a measure of physiological development. However, under certain treatments the two factors (leaflet number and area) are not necessarily highly correlated and it will be suggested that different processes may be involved. Njoku (1956) has postulated a "lobing" substance to be present in the leaves of Ipomoea. The expression of this substance is largely independent of leaf area. A similar mechanism may be present in peas.

Went (1938) was the first person to suggest that two substances are responsible for the increases in leaf area. These he called phyllocaline and caulocaline. The former is responsible for growth of the leaf lamina and the latter for midrib and vein growth. Caulocaline is closely associated with auxin. Went also postulated that leaflet and stipule growth are governed by different growth factors. This at first sight seems surprising, since both structures are laminate and photosynthetic. In only one experiment described in this thesis were the stipules and leaflets affected differentially by treatments applied. It is suggested that this may be a secondary treatment effect. However Went found that while yellow and blue light favour leaflet growth, red favours stipule growth, so the possibility of two factors being involved is not ruled out. Phyllocaline is stored in the cotyledons and produced by the leaves in light conditions only (Went 1938). Went used young etiolated seedlings, whereas the plants used in the experiments to be described were always green and of various ages.

The nature of phyllocaline is still obscure, although it seems fairly certain that auxin is not directly involved. Pea diffusates have been used as a source of leaf growth factors. Bonner, Haagen-Smit and Went (1939) found that activity of diffusates varied with variety of pea used. They evolved a bio-assay technique using actively growing leaf discs. Bonner and Haagen-Smit (1939) tried to find out the active principles in pea diffusates. They found that adenine could reproduce the diffusate effect in part using Raphanus leaf discs as a test object. The increase in wet weight of discs were not very great (about 10%) and have been found difficult to repeat in this laboratory (Sprent unpub.). Galston and Hand (1949) quote Bonner as having isolated hypoxanthine as the active principle, but little further work appears to have been done using this substance. Bonner and Bonner (1940) found that adenine increased leaf growth in intact Cosmos plants, but Kruyt and Veldstra (1947 a and b) could not confirm these results. De Ropp (1945) found no effect of adenine or pea diffusate on isolated rye leaves.

Many workers have found that adenine stimulates general growth in plants. Galston and Hand (1949) found that adenine can promote root, stem and leaf growth in etiolated pea epicotyls and suggested that it may be a factor common to all the "calines" proposed by Went (1938). It is interesting in view of more recent papers to note that the effect on epicotyl growth and root initiation was only shown in the presence of indole acetic acid. Guthrie (1941-2) found that adenine was active in breaking bud-dormancy.

Whether or not other purines can show similar effects to adenine is not certain. Bonner and Bonner (1940) found that uric acid could have a similar effect on Cosmos. In most cases guanine has been found inactive, and this seems to suggest that adenine may not act as additional nucleic acid substrate. The interconvertability of purines varies between organisms. For example, Lactobacillus casei readily interconverts adenine and guanine even when both are present (Bulis et al.1951). This does not normally occur in rat tissues (Reichard 1949). Some organisms e.g. Hemophilus parainfluenzae require

a mixture of purines for growth, while single purines may be toxic (Herbst and Snell 1949).

The mode of action of adenine is still not full understood. Wangermann and Lacey (1953) found that it increased cell size but not cell number in Lemna. In some organisms it may act through riboflavin, as it has been shown to be a procurser of this substance in Eremothecium (McNutt 1954). The effect on general growth suggests that it may act through nucleic acid metabolism, but in some organisms at least, the sugar radical of nucleosides is added before the purine ring is closed (Fry 1955). In these cases the sugar combines with 4-amino-5-imidazole carboxamide. Giri and Krishnaswamy (1957) found that germinating Phaseolus radiatus seedlings (green gram) can metabolise this substance and further work on these lines may elucidate the problem of purine metabolism in plants. Recent work by Skoog and his school has shown that the effect of adenine on higher plants is probably through kinetin (Skoog and Miller 1957).

Kinetin is one member of a group of substituted amino purines to which the general term "kinin" is applied. These substances were first isolated from autoclaved ribose-nucleic acid (Miller et al. 1955), and promote cell division. Skoog and Miller (1957) in a survey of their work on tobacco callus cultures, stress the interdependence of kinetin and indole acetic acid (IAA). The concentrations required are very different, for example optimum results were obtained with 15,000 molecules of adenine and one molecule of IAA. The concentration of kinetin required for stimulating the growth of different organs varies. Skoog and Miller stress the importance of a balanced system for growth, rather than the presence of one specific substance. In this way, hydrolysed casein stimulated growth in some of their cultures in the presence of kinetin and/or IAA. This necessity for balanced systems was found in experiments to be described here and it is suggested that the growth-factor requirements found by different workers for the same processes (e.g. leaf expansion) may not necessarily be the same. Different experimental conditions may cause different components of the system to become limiting.

Adenine has never been found to reduce leaf expansion, it either has no effect or a stimulating one. This is also true of red light, which usually stimulates leaf expansion, the effect being reversed by far-red light. Kinetin can cause a similar increase in area of bean leaf discs, but the effect could not be reversed by far-red light and kinetin could not be replaced by adenine (Miller 1956). The kinetin effect is fairly specific and the kinins can be grouped according to their ability to reproduce the red light effect (Liverman and Scott 1957).

The whole question of the effects of adenine, kinetin and red light on leaf growth is still open. It seems that adenine is not necessarily phyllocaline as has been proposed. The experiments to be described here show only that adenine causes a general stimulation of growth.

It has already been stated that tallness in peas is the expression of the dominant La gene, plants having la la being dwarf. There are, in addition, 2 gene loci which modify the expression of La. These are Cy_1 and Cy_2 (terminology of Lamm 1937, see Lamm 1947 for comparison with other terminologies). One or both dominants are necessary for the normal expression of the La locus. Two recessive mutants of Cy_2 are known, cy_2^c and cy_2^s. The genotype $cy_1\ cy_1\ cy_2^c\ cy_2^c$ is known as "cryptodwarf" and results in semi-tall plants. The combination $cy_1\ cy_1\ cy_2^s\ cy_2^s$ has been called "slender" by deMan (1927) and produces plants with very long thin internodes. DeMan suggested that the dominants Cy_1 and Cy_2 suppress internode growth, except in the presence of La, (Cy_1 being slightly stronger in its action than Cy_2). Thus plants recessive for both loci will be tall regardless of the La component (de Haan 1927). In normal growth and in its response to Gibberellic Acid (GA) (see Ch.4) the cy_2^c allele seems to be intermediate in effect between Cy_2 and cy_2^s. Brian (1957) has suggested that the action of GA on length growth in peas is to release the inhibiting action of Cy_1 and Cy_2. He tested the "slender" genotype and work reported here on "cryptodwarf" confirms his hypothesis. Dwarf peas treated with GA are very similar to "slender" peas in length growth in their long peduncles and occasional abortive flowers (de Haan 1927), and in the larger number of longer cells in each internode (de Haan and Carter 1936).

Radley (1956), isolated GA like substances (natural gibberellins) from tall and dwarf varieties of peas, but did not say whether the amounts differed between varieties. Phinney et al. (1957)have found natural gibberellins in developing pea seed at all stages. Went (1938) stated that caulocaline (which has a similar, though smaller, effect as GA) is produced by the pea roots and is not stored in the cotyledons. These two papers seem to be in disagreement, but the connecting link may be provided by the work of Mitchell et al. (1951), who found that length growth substances occurred in very young bean seed. It is possible that natural gibberellins are only found in the green seed as examined by Phinney et al. and not in the cotyledons after the dormant period, as used by Went. Ritsel (1957) has discussed the time of occurrence of natural gibberellins in higher plants. Evidence presented in this thesis suggests that no great quantity of length hormone is present in the mature seeds even of tall varieties. Went's caulocaline was probably closely connected with IAA and not GA. In view of the recent suggestion of Heath and Clarke (1956) that IAA may act only after chelation, the vital role of the roots in Went's seedlings may have been to provide the necessary metal ions, by adsorption from the surrounding medium. However, de Hopp (1946) found that adsorption contributed only a part of the effect of roots on stem growth in rye. Tissue union was required to give full effect. This suggests that some complex (or unstable) substance is involved.

Physiological differences between tall (_la_) and dwarf (_la_) peas have been fairly intensively investigated. Van Abrens (1953) obtained a small effect (compared with GA) of IAA in increasing the height of dwarf peas, whereas IAA had no effect on tall plants. He was unable to relate the differences obtained with any aspect of auxin metabolism. Brian and Hemming (e.g. 1957a) have found smaller effects of IAA on dwarf peas and their results have been confirmed in this laboratory (see chapter 4). The fact that isolated internodes respond greatly to IAA, whereas whole plants do not, has always been an intriguing problem to physiologists. Brian and Hemming (1958) have recently put forward a very interesting proposal to explain this problem. They suggest a three

factor system, involving auxin, one or more natural gibberellins and an inhibitory system.

Stem growth is readily affected by physical environment as well as by chemical treatments. Light probably has the most important effect. It is well known that etiolated plants are taller than green ones. This may be closely connected with the effect of GA. The effect of Gibberellins on Alaska peas has been described by Lockhart (1956) as reversal of light inhibition of growth. Lockhart further suggests that the site of natural GA-factor production is the stem apex (see also Lockhart 1957a). In bean seedlings Lockhart (1957b) found that 2-5 minutes exposure to red light was necessary to obtain an effect of GA. Whether this fact implies a relationship between GA and kinetin metabolism is not known. Brien and Herring (1957b) could find no such relationship.

Lack of light stimulates internode elongation in peas (see above), but pea plants grown in long days are taller than those grown in short days. This is true for both tall and dwarf varieties as will be shown in Chapter 4. This may be a result of a different mechanism from the hormonal one e.g. increased carbohydrate production.

In the preceding pages, factors affecting flowering, leaf and stem growth in peas have been discussed. Some of these have been connected genetically as pleiotropic effects of the one locus. The pleiotropism probably arises from physiological correlations, since known substances (IAA, GA, kinetin) can effect more than one aspect of growth. In the work to be described all three growth processes mentioned have been measured, as this was considered to lead to a more balanced concept of the growth and development of Pisum.

One problem that frequently arises during the type of experiment which has been carried out, is the difficulty of separating effects caused by nutritional differences and those caused by growth substances. This is particularly true where the experiment involves cotyledons, since these organs are a known source of both food material and growth substances. It will be suggested that both alternatives contribute

to the general growth system and under some circumstances it will be shown that the two processes can be separated.

ii Materials and Methods

PLANTS USED The four commercial varieties Alaska, Telephone, Greenfeast and Massey have been used for most experiments. The last two, being dwarf varieties, were most easily handled and were used whenever possible. Where other varieties and genotypes were used they will be described.

GLASSHOUSES Plants have usually been germinated in moist vermiculite or vermiculite: gravel mixture and then planted out into potting soil in one of several glasshouses, either in boxes approximately 50 x 35cm. surface area and 17cm. deep, or in beds. Natural daylight has been supplemented where required with 250W Mazda horticultural lamps, or a combination of 40W fluorescent strips and incandescent globes.

In the earlier photoperiodic experiments the main bed in one glasshouse was divided into 2 and one half was covered with light proof blinds when required, while the other received supplementary light. The main disadvantages of this system are that the different positions of the two compartments may result in temperature and natural daylight gradients which cannot be removed by experimental design. An especially designed glasshouse was used for later experiments involving photoperiodic treatments. In these experiments plants were grown in boxes which fitted into metal trolleys, eight boxes per trolley. Each bay of the glasshouse had four trolleys (on rails) from which a random pair was taken for removal into short days when necessary. The short day compartment was sealed by blinds which fell into slots in the rails (see fig. 4.1). Fans maintained an airflow through both compartments. In this way all plants received equivalent amounts of natural daylight and temperature variation between short and long days was reduced.

APPLICATION OF CHEMICAL SUBSTANCES. Various methods were tried, some of which will be described in detail later. Sub-epidermal injections were found to be unsatisfactory as insufficient liquid could be administered. A pea plant does not have a hollow pith suitable for receiving injected solutions until its growth pattern has become almost fixed. Both the soaking of cuttings in chemical solutions and application of drops of solution to the shoothed, cut stump of the main shoot (development of cotyledonary

axillaries used as test) had certain advantages. Plants were also grown in semi-sterile liquid culture and on sterile agar media. In addition, several experiments were made in which chemicals were applied in solution to the leaves as described by Brian and Hemming (1955) in their work on Gibberellic Acid. Experiments designed to test the interaction between gibberellic acid (GA) and vernalization, as well as those involving early maturing varieties, made it essential to apply chemical substances prior to germination. The following method of seed treatment was therefore devised for use with chemical substances (e.g. GA) soluble in absolute alcohol (or alcohol:water mixtures). A fine capillary was attached to a burette so that drops of 10µl were obtained. These were easily applied individually to the dry seed and the alcohol evaporated readily near a radiator. The dry seeds were placed in saturated vermiculite. In this way each plant received a known amount of chemical substance. As water passes only from the vermiculite into the seed the substance cannot be washed off. The amount lost by diffusion away from the seed was very small. The method was most easily applied to wrinkled seed where the solution was placed on the opposite side of the seed to the "embryo" to avoid damage. Round seed (e.g. Alaska) had to be treated more carefully. Seeds treated with GA in this way gave a marked response as soon as germination began. In contrast with the results of Bukovac and Wittwer (1956) no effect was noticed on the germination time. This difference may be due to different dosages.

Scoring. Methods of scoring were largely those of Paton and Barber (1955) and Paton (1956). The node of first flower was numbered from the base using the cotyledonary node as 0. An unfolded leaf was taken to be one in which the leaflets had grown clear of their enshoathing stipules. A leaf was said to be expanded when the two halves of a leaflet had begun to open out. In most cases the measure of node of first 4-leaflet leaf was replaced by node at which more than two leaflets occurred, as this seemed the more stable factor. Many plants have one or more nodes with three leaflets.

Leaf Area. It was decided that graphical or planimeter methods were too tedious for large numbers of measurements and therefore a photoelectric method was devised.

The sensitive surface was an "Ilaf" Selenium Photocell 40 x 60mm. selected for uniform sensitivity over its surface, by measuring a standard area at different places on the surface. For protection, a thin piece of glass was cut to the rim of the cell and was sealed to the rim with clear nail varnish. The cell was connected to a microammeter which had a 1000 ohm radio potentiometer connected across its terminals to permit alterations of the sensitivity range and to allow adjustments to be made when there were slight variations in the mains voltage. A switch was placed between the photocell and the ammeter. The apparatus was mounted in a wooden case as shown in Fig.1.2 the positions of the components being chosen to give maximum comfort and ease of reading.

The instrument gives best results when used in a darkened room, where the only light is that required for operation (e.g. a 200W globe at a suitable fixed distance from the cell such that the light rays were nearly parallel). The globe was cleaned of writing, (maker's name etc.) to ensure even illumination. When in use the instrument is switched on and the ammeter reading adjusted to 100 μamps using the potentiometer. The specimen is then placed directly on the cell and the ammeter reading taken. The reduction in ammeter reading is directly proportional to the area of the specimen. The instrument was calibrated with leaf discs of known area and a table made. Ammeter readings can then be directly converted to area. For measuring small areas a piece of glass painted white and occupying about one half of the sensitive area is placed at one end of the cell. The ammeter was then re-set to 100 μamps with the potentiometer, and the instrument recalibrated. Thus as long as the cell was big enough to measure the largest leaves it could be converted for use with smaller areas, giving the same percentage accuracy.

The ammeter reading is checked between specimens and returned to 100 if minor voltage fluctuations have altered it. The variation is seldom greater than one μamp and usually less. It has been found that in the leaves measured there was a negligible variability in transmitted light.

A comparison of methods of measuring leaf area is shown in table 1.1.

Table 1.1

Comparison of Three Methods of Leaf Area Estimation

The following figures were all taken using pea leaf discs of known area 78.57mm^2. Values for 24 discs were as follows:-

1. Using Photoelectric cell:
   80.5, 80.5, 72.5, 72.5, 72.5, 80.5, 72.5, 72.5, 72.5,
   80.5, 80.5, 72.5, 72.5, 72.5, 80.5, 72.5, 72.5, 80.5,
   72.5, 80.5, 72.5, 72.5. Mean value 75.8 Variance of mean 16.2.

2. Using planimeter, tracing drawings of discs:
   80, 85, 90, 75, 80, 80, 80, 81, 90, 89, 75, 80, 78, 87,
   80, 92, 91, 85, 75, 87, 80, 82, 80, 91. Mean 83.0, Variance 29.4.

3. Counting squares on graph paper (using same tracings as method 2).
   86, 85, 85, 86, 79, 83, 79, 76, 80, 87, 94, 89, 91, 85,
   86, 89, 91, 97, 93, 89, 89, 85, 85, 87, Mean 86.5, Variance 24.5.

It can be seen that methods 2 and 3 involving tracing gave too high an estimate. The variance of all three means is of the same order. The variation in method 1, which has only 2 different values, is due to the fact that the microammeter could only be read to 0.5 microamps. The two values obtained differ by 0.5 microamps. Since we usually work with larger areas and this error remains constant, the percentage error becomes less. The readings given above were taken using the whole cell and the accuracy could have been increased by using half the sensitive area. The error obtained here, therefore, is the largest that is likely to be encountered.

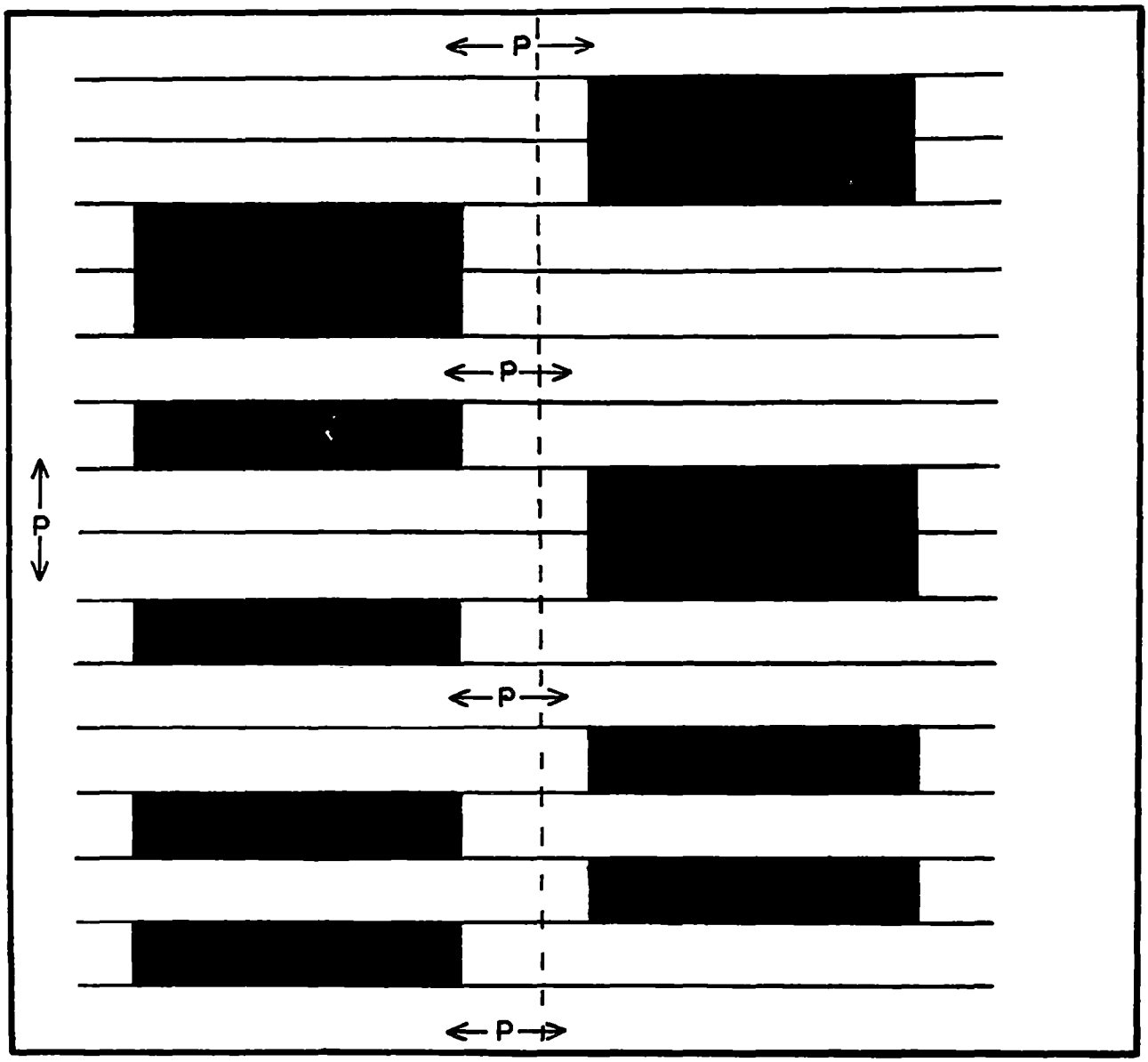


Fig. 1.1. Diagram of the glasshouse used in photoperiodic experiments. P indicates pathway. The solid black areas are a typical random set of truck positions during the night period. The vertical dotted line represents the positions of the blinds, the area to the right of this being the dark compartment, and to the left the light compartment. Horizontal black lines represent the rails upon which the trucks are moved.

Fig. 1.2. General view of the photoelectric device used for measuring leaf area. The Selenium cell on which leaves are placed is on the left.

III Abbreviations used in text.

F	Node of first flower (numbered from base)
U	Number of unfolded nodes i.e. up to and including the last leaf which is free from its ensheathing stipules.
E	Number of expanded nodes i.e. those in which the two halves of a leaflet have separated.
Ca	First node with more than two leaflets.
Cb	First node with four leaflets.
Cbs	Stable node of four (or more) leaflets i.e. after any reversion.
% R	Percentage plants in which reversion from a higher to a lower number of leaflets takes place.
ALx	Area of leaflets at node x (cm^2)
ASx	Area of stipules at node x (cm^2)
L	Total length of main shoot (cm)
L_{a-b}	Length of shoot from nodes a to b. (cotyledonary node is referred to as node 0) (cm.).
Th_x	Leaflet thickness at node x (mm.).
G	General growth on arbitrary scale, taking into account number of dead leaves, leaf size, length etc.
GA	Gibberellic acid
IAA	β indole acetic acid.
SD	Short Day
LD	Long Day
V	Vernalized or Vernalization
UV	Unvernalized (sometimes reduced to U where the meaning cannot be confused with number of unfolded nodes).

A diagram to show the use of some of the abbreviations is given overleaf.

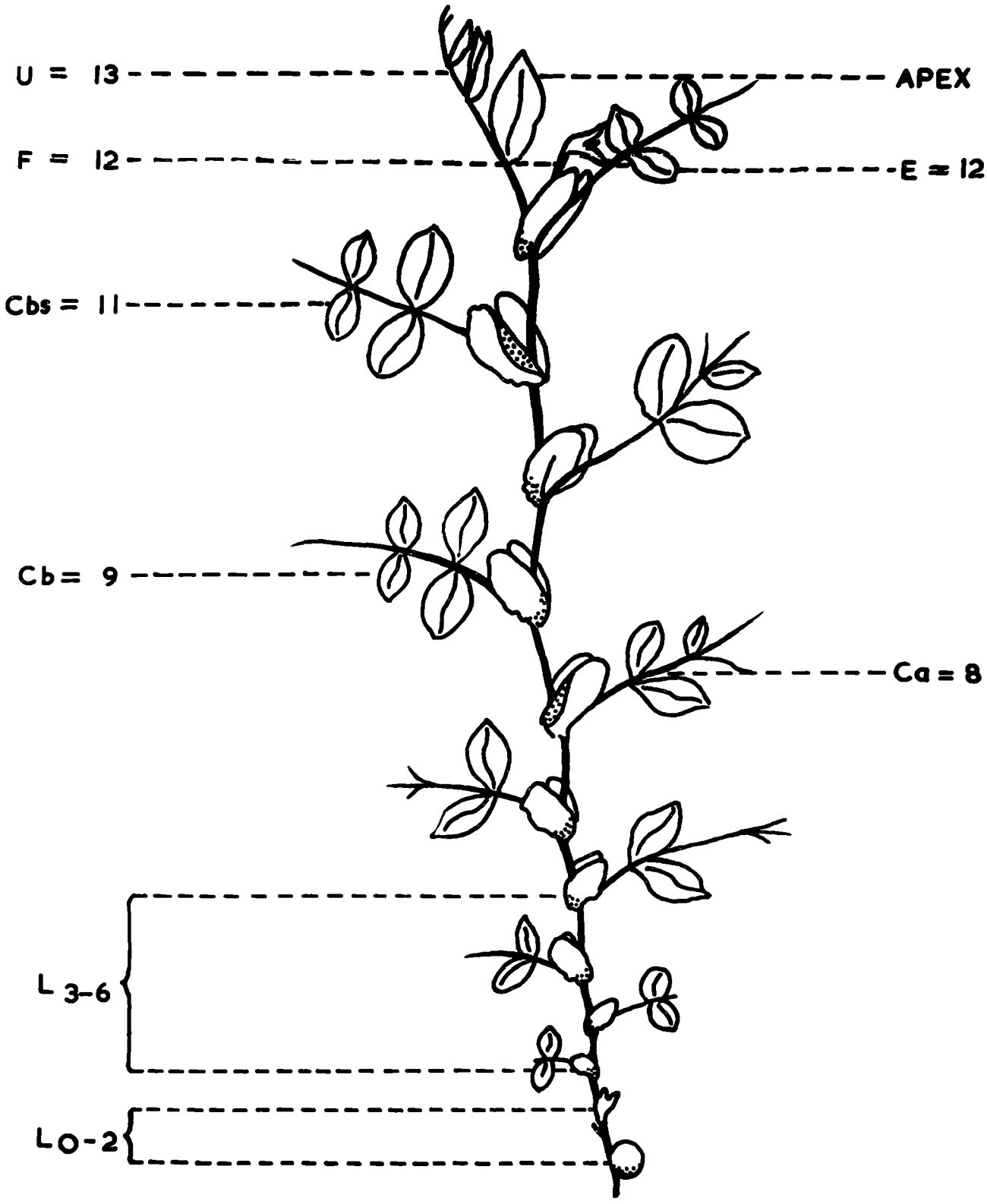


Diagram to illustrate the use of abbreviations in measuring different variables. For explanation of the symbols see previous page.

CHAPTER 2.

COTYLEDONS AND GREEN LEAVES AS SOURCES OF GROWTH FACTORS

1. Introduction

The important influence exerted by cotyledons on plant growth has been noted by many workers. In peas, cotyledons are the major source of nutrient for the young seedling. In addition, they provide growth substances such as the hypothetical rhizocaline and coulocaline (Went 1938). Working with etiolated plants, Went found no effect of cotyledons on stem length. Highkin (1955) has claimed that pea cotyledon diffusates contain flower promoting substances, thus supporting the work of Haupt (1952) using early varieties. Barber and Paton (1952) postulated that late pea varieties contain colysanthin in their cotyledons. Experiments to be described here confirm Barber and Paton's theory and the possibility that early pea varieties contain a flower promoting substance. Henrickson (1954) obtained a higher proportion of flowering sunflower plants when parts of the cotyledons were removed, but not when entire cotyledons were removed. Thus there appears no doubt that vital substances pass from the cotyledons into the growing seedling. It is difficult in some cases to decide whether these substances are nutritional or hormonal.

The part played by green leaves in the development of peas has been less well defined. In late varieties they appear to be intimately connected with the photoperiodic response. Paton (1956) has postulated that colysanthin passes from the cotyledons into the lower leaves, where, under long days, it is either destroyed or converted into flower hormone. Short day conditions prevent the destruction of, and promote further formation of colysanthin in the leaves. In long days leaves of all varieties produce flower promoting substances. Leaves are also sources of leaf growth substances (Went 1938).

In this chapter, the effects of removing cotyledons and green leaves will be described. It will be seen that the cotyledons exert a more profound effect on the growth of peas than a comparable amount of green leaf tissue.

ii. Transport of Growth Factors from the Cotyledons.

Paton and Barber (1955) removed cotyledons from pea varieties at 10 days after germination, (at the same time they performed their grafts). They found that cotyledon removal had no effect on Massey but led to a reduction in node of first flower (F) in Greenfeast. This reduction was thought to be due to the removal of colysanthin. Pea cotyledons have also been shown to contain leaf growth substances (Went 1938), therefore an experiment was planned in which the time course for removal of growth substance from the cotyledons was investigated.

Experiment 2.1. Massey and Greenfeast peas were germinated and planted out into the greenhouse in rows which were alloted at random to the following treatments:- cotyledons removed at 4, 6, 8, 10, 12 and 14 days after germination, and a control series of intact plants. The plants were grown under natural long days (16 hours) and were scored for F, first node with more than 2 leaflets (Ca), shoot length (L) and number of unfolded nodes (U).

The general level of F in Greenfeast plants was higher than usual for long day conditions. This was probably due to low light intensity as the plants were grown in the shade of a wall. Figure 2.1 gives these results, together with the standard errors of the treatment means. The quadratic regression line is highly significant. The data for Ca in Greenfeast are also shown in Figure 2.1, and are seen to show the opposite trend to F. The Ca trend is almost completely linear (see table 2.1 for regression analysis). A sigmoid curve would probably fit the biological facts more closely, since the removal of cotyledons later than 14 days after germination would presumably have no effect on Ca. Greenfeast data for L and U are given in Figure 2.2 and Table 2.2 respectively.

The number of surviving plants in Massey was rather low, but the main results obtained are shown in table 2.3. The treatments had little effect of F, except that removal of cotyledons at four days after germination may have removed a substance which promotes flowering. Most of the apices with earlier (more severe) treatments were dying at the time of scoring.

Discussion: Both U and L are measures of general vegetative growth. They were only significantly affected by treatment when cotyledons were removed on or before 6 days after germination. This contrasts with the effect of treatment on Ca and F in which the 8 day (and also for F the 10 day) treated plants were significantly different from the controls. Thus under these conditions, it appears that seedlings can manufacture the bulk of their own food supply after one week from germination, while at this stage they are still partly dependent on the cotyledons for growth substances. This indicates that the figures obtained for Ca and F cannot be explained completely as a secondary

result of changes in vegetative growth. The results for F are in complete agreement with the theory of Harbor and Paton (1952). Under the conditions of the present experiment, colysanthin passes into the plumule during the first 12-14 days after germination. Later experiments have shown that deviations from this range are rare. The results for Ca support Went's (1938) proposal that leaf growth substances are present in the cotyledons, but the length data do not support his suggestion that cotyledons have no effect on stem growth. However, his experiments were with etiolated plants of a tall variety (Alaska) and were of a much shorter duration. Kruyt (1952) observed effects with the variety "Groene Ionte" similar to those obtained in the present experiment, but gives no details of results. The Massey data for Ca and L is similar in trend to that for Greenfeast, although the treatment effect on Ca is much more severe.

The fact that two varieties show similar effects of treatment on vegetative growth but different effects on flowering, further suggests that colysanthin is a true flower inhibitor and does not act by altering vegetative growth. Harbor (1958) has suggested that the dominant Sn gene may enable plants to convert flower hormone into colysanthin. This implies that sn plants contain a flower hormone as Haupt (1957b) has shown for some varieties, and could explain the significant delay in F when the cotyledons were removed from Massey at 4 days after germination (table 2.3).

During the analysis of this data, it was noticed that the variability of the plants whose cotyledons were removed was less than that of those left intact. This effect has been found previously by Kruyt (1952). Since the plants were not grown under sterile conditions, the cotyledons rotted at different times, resulting in variability in the supplies of growth factors. When cotyledons are removed, this variability is eliminated.

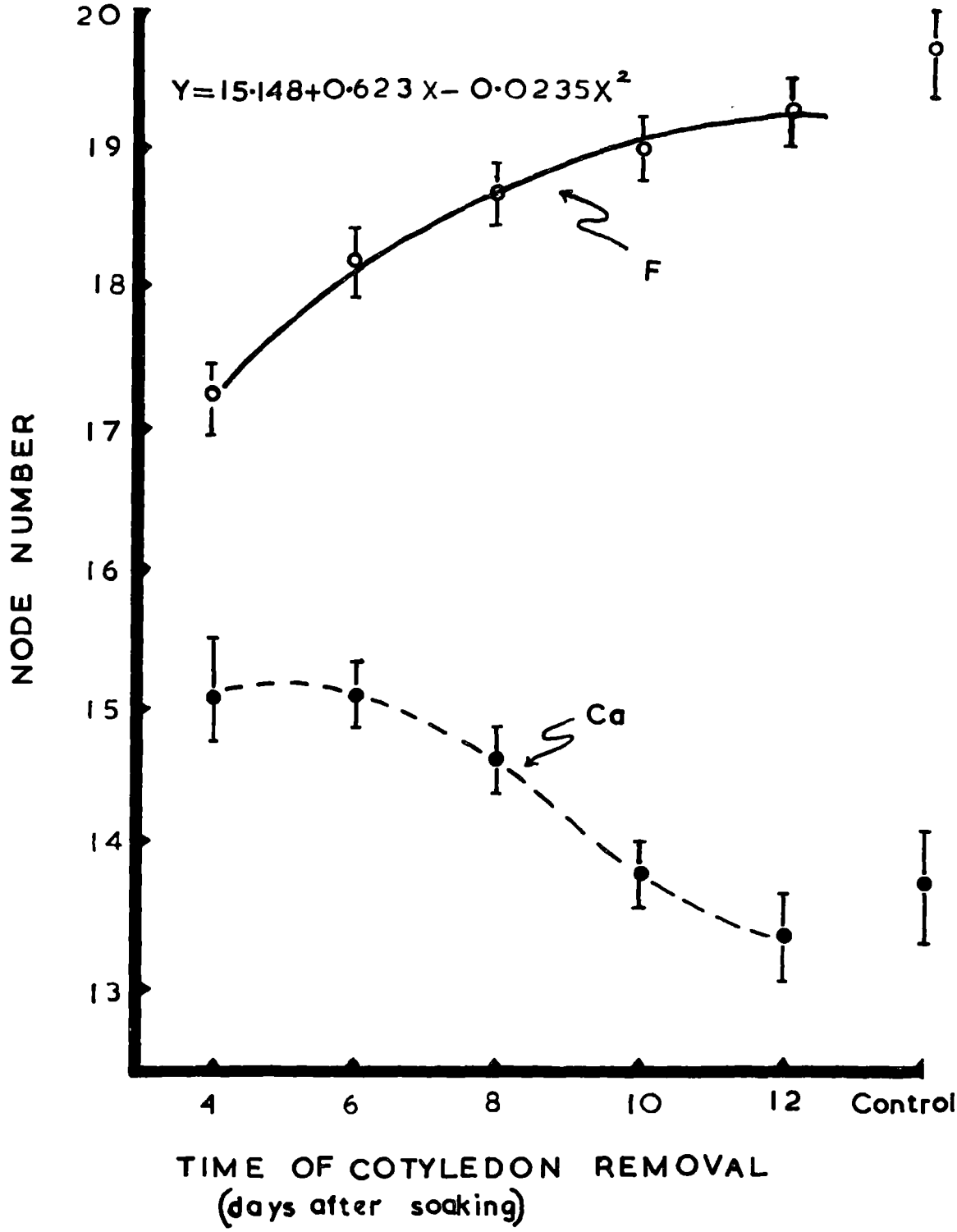


Fig. 2.1. Effect of removal of cotyledons on node of first flower (F) and node at which leaves with more than two leaflets are first produced (Ca) in Greenfeast. Treatment means and their standard errors.

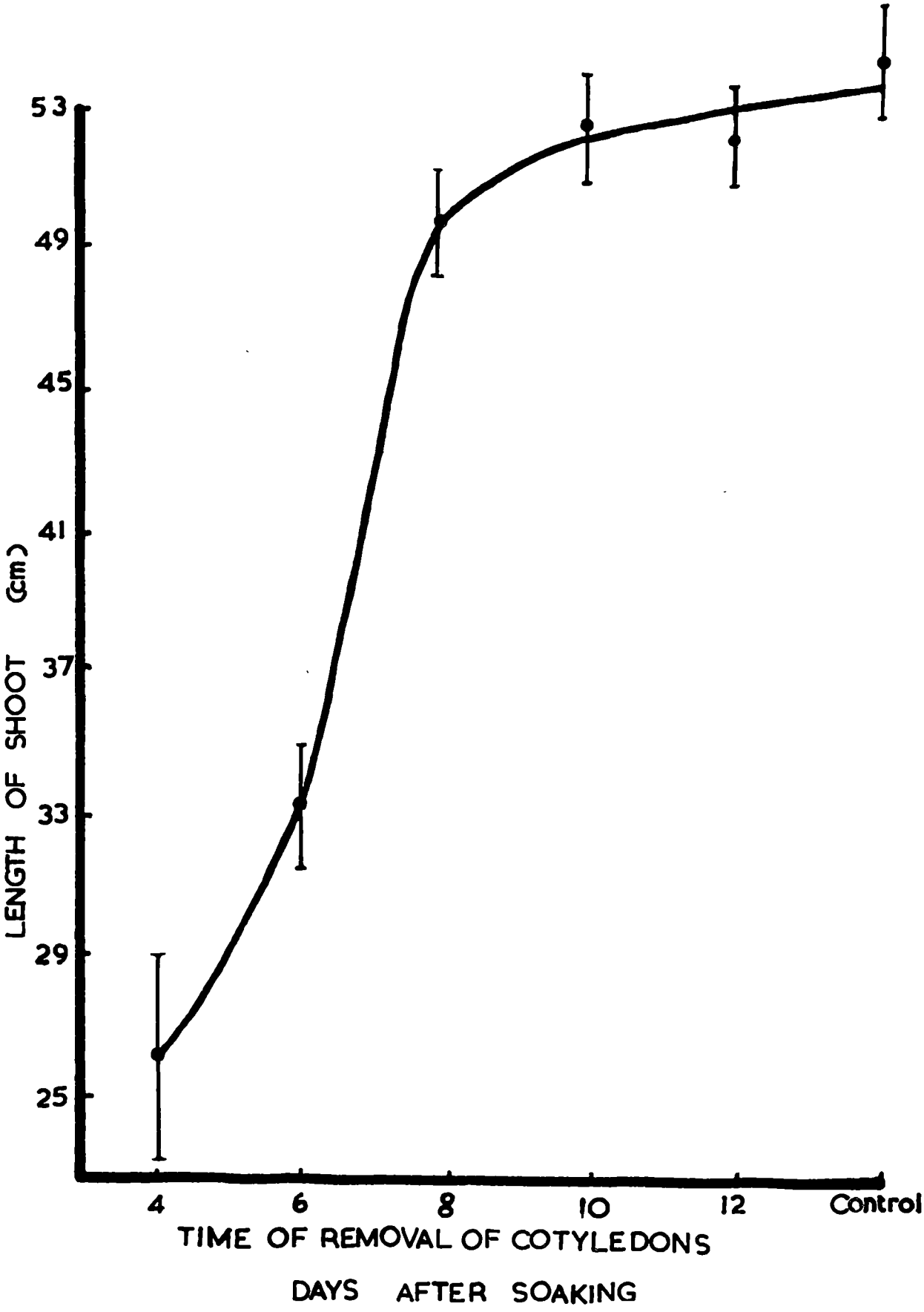


Fig. 2.2. Effect of removal of cotyledons on length of main shoot (L) in Greenfeast. Treatment means and their standard errors.

Table 2.1

Linear regression analysis of Cn on time of removal of cotyledons.

Treatment means shown in fig. 2.1.

Effect	Sum of squares	Degrees of freedom	F ratio	Significance level
Regression	41.38	1	27.02	0.1%
Deviations	2.06	3	0.45	n.s.
Error	145.80	95		

Table 2.2

Number of expanded nodes in Greenfeast peas with cotyledons removed at different intervals after germination. Natural LD (About 16 hours).

Age of plant when cots. removed (days)	N at time of scoring
4	17.50
6	18.79
8	19.79
10	19.88
12	19.80
Control	20.05

Table 2.3

Effect of removal of cotyledons on growth of Massey peas. LD (about 16 hrs.)

Results and standard errors

Age of plant when cots. removed (days)	Ca	F	L	n
4	none formed	11.33 ± 0.36	15.00 ± 1.73	3
6	none formed	9.35 ± 0.13	16.29 ± 0.54	17
8	⩾11.00	9.07 ± 0.08	20.64 ± 0.89	14
10	10.26 ± 0.19	9.32 ± 0.10	21.26 ± 1.09	19
12	10.19 ± 0.19	9.14 ± 0.15	20.00 ± 0.67	21
Control	10.38 ± 0.22	9.23 ± 0.12	22.00 ± 1.07	13

Table 2.4

Relation between age and development of treated peas July/August. Natural SD (about 10 hrs.)

Age in Days	MASSEY			GREENFEAST		
	No. of nodes visible	No. exp. internodes	L mm.	No. nodes visible	No. expanded internodes	L mm.
7	2	1	8	2	1	10
10	3	2	10-15	3	2	10-15
14	5	3	45	4	3	30
18	6	4	50-60	5	3	40
22	6	5	70	5	4	50
27	7	5	100-160	6	5	80-90

iii. Effect of Cotyledons and Roots on Cuttings and Cotyledonary Axillary Shoots.

The quantitative relationship between cotyledons and various aspects of growth has been established in section ii. Since colymanthin passes from the cotyledons into the plumule, cuttings of the latter should contain a quantity of inhibitor which is proportional to the time interval between germination and cutting date. The amount of inhibitor remaining in the cotyledons varies correspondingly, a fact which should be reflected by the flowering node of the cotyledonary axillary shoots. Experiments to confirm this hypothesis will be described in this section, together with some to investigate the possible effect of roots on growth.

Experiment 2.2. This experiment was originally carried out by Barber in California, using the varieties Massey and Telephone. Greenfeast and Massey were used for the present experiment. The seed was germinated in the usual way and seedlings planted into a greenhouse bed. Cuttings were taken at intervals of 7, 14, 18, 22 and 27 days after germination and planted into boxes. One cotyledonary axillary was allowed to grow from each of the parent plants. A control series of untreated plants was also grown. Plants were grown under natural long days (about 14 hours) and were scored only for node of first flower (F) and first node with four leaflets (Cb). Table 2.4 (previous page) shows the size and development of plants when cuttings were taken.

Table 2.5 gives the Massey data obtained here and by Barber in California. The very young cuttings died. It can be seen that there is little or no effect of treatment on node of first flower in either experiment. The figures for Cb correspond closely, the early cuttings showing an almost indefinite delaying effect which is probably due to poor growth. The data for Massey axillaries are shown in table 2.6. The later treatments proved lethal. The node of first flower was again very uniform over all treatments. The later node of first 4 leaflet leaf in the axillaries corresponding to the later cuttings may be attributed to depletion of either food reserves or growth substance (or both) from the cotyledons.

Table 2.7 gives the results for Greenfeast cuttings and for comparison Barber's Telephone data. The only Greenfeast cuttings which flowered at as high a node as the controls were those taken at 18 days. Those taken earlier and later than this point showed a lower value of F. The Telephone data do not correspond. The 4 leaflet leaf figures show similar trends in Greenfeast and Telephone and in the former the values parallel those for F. Many of the Greenfeast cuttings did not at first maintain the four leaflet leaf condition, but instead reverted to values of 2 or 3 before becoming stabilised at 4. Table 2.8 shows a comparison of first (Cb) and stable (Cbs) nodes of four leaflet leaf. The percentage reversion rose as the cuttings were taken later.

Table 2.9 gives the results for Greenfeast and Telephone axillaries. The node of first flower in Greenfeast shows a steady fall as cutting age is increased. This effect is not shown in Telephone. As cutting age is increased, the node of first 4 leaflet leaf is increased in both varieties.

Experiment 2.3. This experiment was designed to test the effects of roots on growth and development of Massey and Greenfeast peas containing different amounts of the growth factors present in their cotyledons. Plants were decapitated at 4, 6, 8, 10 and 12 days after germination. One half of the plants also had their cotyledons removed at the time of decapitation. Both cotyledonary axillaries were allowed to grow and when they had reached a height of 1-1.5cm. (about 14 days after decapitation) one was removed and grown as a cutting. Where the two axillaries were of unequal size alternate large and small ones were used as cuttings. Cuttings were only taken from plants with intact cotyledons. This procedure resulted in three series of plants per variety:-

(a) Axillaries + cotyledons + roots
(b) Axillaries - cotyledons + roots
(c) Axillaries - cotyledons - roots

The experiment was carried out under natural long days (16 hours) and the plants were grown in boxes. They were scored for node of first flower (F), first node with more than 2 leaflets (Ca) and shoot length (L).

The figures for F in Massey are shown in table 2.10. The number of survivors was very low and few plants produced leaves with more than 2 leaflets.

Greenfeast axillaries with cotyledons and roots should be comparable to those in the preceding experiment. A comparison of the two sets of data is given in figure 2.3. It can be seen that the figures for Ca and Cb are very similar, but in the present experiment there was no treatment effect on flowering, i.e. the results are similar to those obtained by Barber for Telephone.

Figures for Greenfeast axillaries without cotyledons and with roots are shown in table 2.11. Very little treatment effect is shown. Axillary cutting data for F and Ca are shown in figure 2.4. There is a similar trend in both variables. A comparison of Ca and Cb figures for the cuttings is given in table 2.12. It can be seen that in this case the two estimates of leaf growth do not entirely correspond.

The L figures for all Greenfeast treatments are given in table 2.13. There is a marked reduction in shoot length as the time of decapitation becomes later in all cases, except where roots but not cotyledons are present.

Discussion. Flowering in Massey showed the usual lack of response to treatment, probably because most treatments were made after flowers had been initiated. In the second experiment, plants with cotyledons tended to flower at a lower node (7.24) than those without (8.04). This confirms the presence of a flower hormone in Massey cotyledons. Massey treatments in which the greater part of the cotyledonary influence was removed (i.e. cuttings taken early or axillaries grown into) showed

a very marked delay in the production of leaves with more than two leaflets. This could equally well be explained as resulting from loss of either food materials or growth substances, or both.

The Greenfeast and Telephone cutting data are not entirely as expected. Both varieties possess the dominant Sn gene controlling late flowering and presumed also to be the gene responsible for the production of flower inhibitor. For this reason the results were expected to be similar in the two varieties. The node of first flower in Telephone cuttings showed hardly any response to treatment. The three groups of cuttings which survived all had a higher value of F than the control (whole) plants. However, the variability in these groups was so large that the differences scarcely reached significance. In the first experiment, Greenfeast cuttings taken between 7 and 13 days after germination flowered at a gradually increasing node number, due to the increasing amount of colysanthin in the plumule. The time taken for the colysanthin to pass from the cotyledons into the plumule was slightly longer than that found in section 4, but this may have been due to the cooler conditions under which the present experiment was conducted. Cuttings taken later than 13 days after germination showed little ability to form roots and their vegetative growth was greatly slowed down. This may account for the lower flowering node of these plants. The effect was probably accentuated by the gradually increasing daylength during the growth of these cuttings. This would have increased the rate of destruction of colysanthin (or stimulated the production of flower hormone, see general introduction).

The values of Cb follow the same trend in Greenfeast and Telephone. In the former there is a close correlation between this variable and F. This correlation is probably misleading, since experiment 2.1 indicated that cotyledons promote the formation of four leaflet leaves and thus cannot have been responsible for the figures obtained in the earlier cuttings. These cuttings, by getting established earlier may have been able to synthesise their own leaf growth substances in time to form four leaflet leaves at nodes corresponding to these in the control plants. The rate

of node formation in these early cuttings was also rather slow and this may have resulted in the factors synthesised by the lower leaves being available to lower nodes than in plants which were growing at a faster rate. The 10-18 day cuttings produced four leaflet leaves at a higher node than the whole plants due to the smaller amount of growth factors from the cotyledons. The 22 day cuttings had enough of these growth factors for four leaflet leaves to be produced at the same level as in the controls. The value obtained for the 27 day cuttings is difficult to explain, since it is significantly below that of the controls. The increase in percentage reversion (table 2.8) with increasing cutting date supports the theory that the node of first four leaflet leaf is established when the later cuttings were taken. The act of taking cuttings deprived the shoot of food substances and thus reversion to a lower number of leaflets per node occured until the cuttings recovered.

The flowering behaviour of the axillaries with cotyledons and rootswas not entirely as expected. There was no effect of treatment on Telephone. In Greenfeast experiment 2.2, only those axillaries corresponding to the 7-18 day cuttings survived. These showed an increase in node of first flower as more inhibitor was removed with the cutting. This effect was not repeated in the corresponding cuttings in experiment 2.3, but this again may have been a result of different photoperiod. The Telephone data are hard to explain, since this experiment was also conducted in short days. It is doubtful whether a true varietal difference exists, since Paton (1956) has shown that Greenfeast and Telephone are similar in their inhibitor mechanisms. It is possible that in the shorter days (8 hours) of the Californian experiment the colysanthin produced by the leaves is greatly in excess of that present in the cotyledons, and the effect of the latter is masked. The pattern of four leaflet leaf development in Telephone, Massey, and both series of Greenfeast cuttings is very similar, although the absolute values are different (figure 2.4). They can be explained by a gradual reduction of growth substance as the plants were decapitated at a greater time after germination. As all varieties tested showed a similar response to treatment it seems

unlikely that they differ appreciably in their content of leaf growth substances.

When cotyledonary axillaries were grown without cotyledons, no effect of cutting date on F or Ca was observed. This is to be expected if the cotyledons are the only source of growth substances for these processes, since cotyledons and plumule were removed at the same time. The results indicate that roots do not have a modifying effect on either flowering or number of leaflets.

The data for Greenfeast axillary cuttings give significant linear regressions of node number on time of decapitation, both for flowering and Ca. In the latter case deviations were also significant. After decapitation these axillaries were in contact with their parent cotyledons for about 14 days. This may have affected their response to treatment. The earlier cuttings may have obtained some colysanthin from the cotyledons, although it is not clear why this should have affected the axillary cuttings and not those with cotyledons and roots. The results for Ca and Cb do not correspond exactly. The latter is fairly uniform, although it may be delayed in the later cuttings. This would suggest a cotyledonary effect. The greater than 2 leaflet leaves may have been formed as a result of a temporary slowing down in the rate of node formation. This hypothesis will be more fully discussed in the next chapter.

The effect of treatment on shoot length in experiment 2.3 can probably be explained on nutritional grounds. As the plants become reduced in size as a result of treatment, their length becomes smaller.

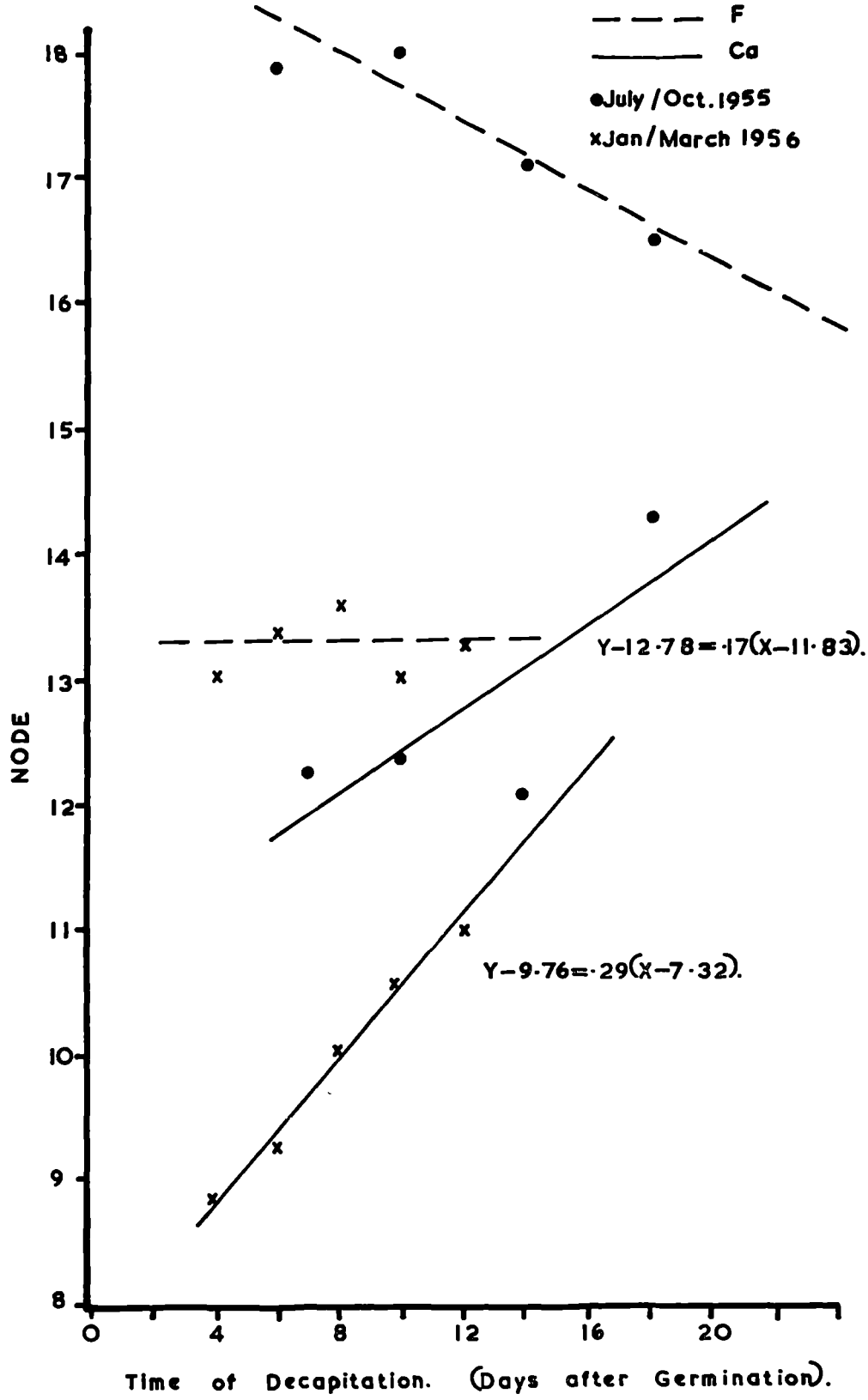


Fig. 2.3. Growth of cotyledonary axillary shoots of Greenfeast peas. Regression lines of node of first flower (F) and first node at which more than two leaflets are produced (Ca) on time of removal of main shoot. Data for two similar experiments conducted at different times of the year under natural day-lengths. The results for F in the Jan/March experiment showed no significant treatment effect and the line was drawn through the mean value for all treatments and parallel to the x axis. All the other lines represent a statistically significant linear trend with non significent deviations.

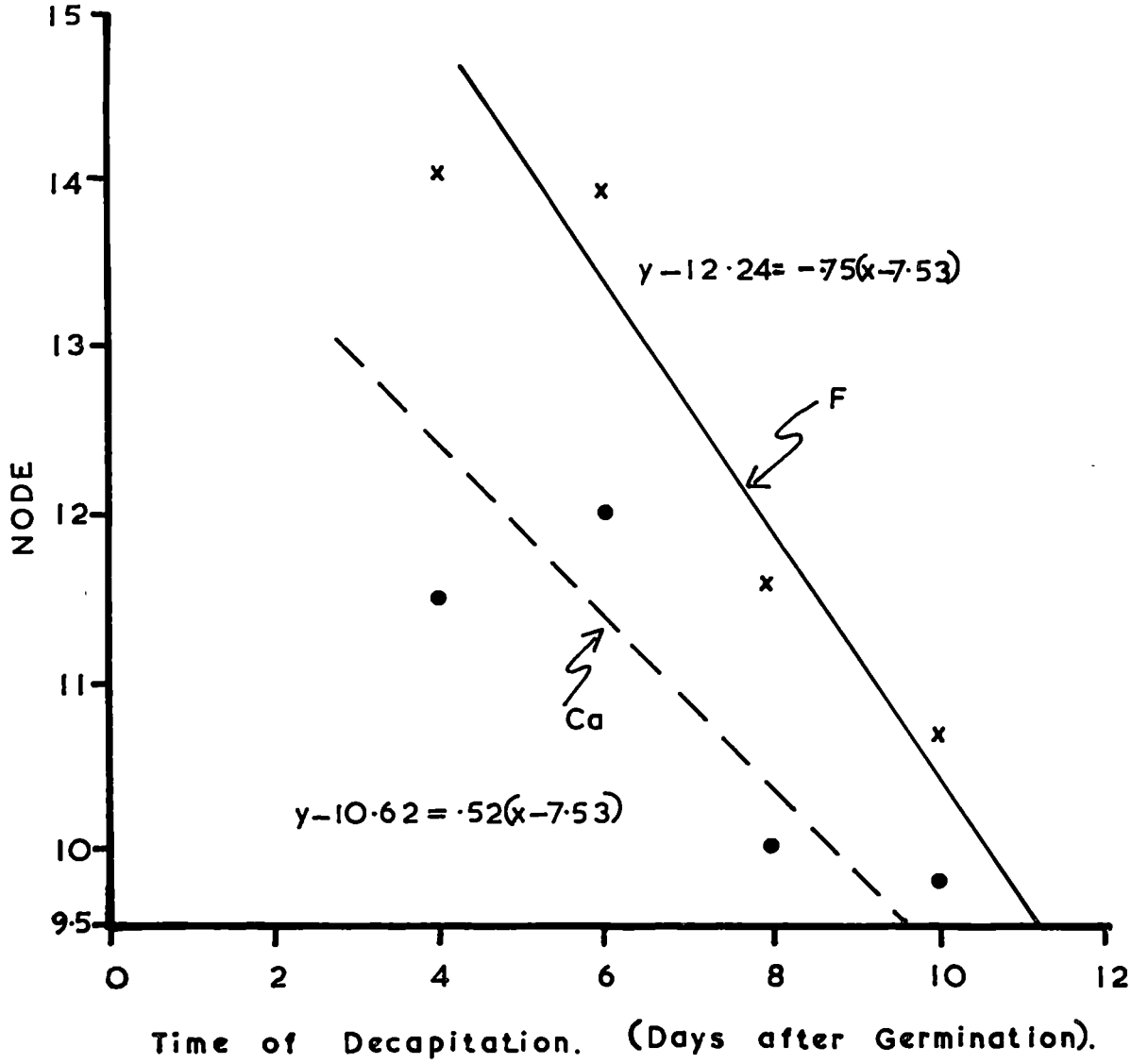


Fig. 2.4. Growth of cuttings of cotyledonary axillary shoots of Greenfeast peas. The shoots grew in contact with their stocks for two weeks before cuttings were taken. Regression lines for node of first flower (F) and first node at which more than two leaflets are produced (Ca) on time of removal of cotyledons. Both linear trends are significant, but for Ca the deviations are also significant at the 5% level.

Table 2.5.

Growth of Massey Cuttings taken at intervals after germination. July/August.

Natural SD (about 10 hrs.)

Results and standard errors.

Age of plants when cuttings taken (days)	F		Cb	
	Exp. A	Exp. B	Exp. A	Exp. B
7	10.11 ± 0.21	10.43 ± 0.30	⩾ 13.35	⩾ 15.0
10		10.10 ± 0.23		⩾ 15.0
11	9.80 ± 0.20		⩾ 10.80	
14		10.00 ± 0.00		11.30 ± 0.21
17	10.00 ± 0.00		10.38 ± 0.18	
18		⩵ 10.00		10.38 ± 0.26
22		10.31 ± 0.17		11.00 ± 0.23
27		10.25 ± 0.16		11.00 ± 0.21
Control	9.75 ± 0.17	10.23 ± 0.44	10.69 ± 0.15	11.00 ± 0.16

Expt. A is that conducted by Barber in California

Expt. B is the Hobart Experiment

Table 2.6

Growth of Massey cotyledonary axillaries from stocks of plants used for table 2.5

Age of plant when cuttings taken (days)	F		Cb	
	Exp. A	Exp. B	Exp. A	Exp. B
2	7.40 ± 0.16		8.30 ± 0.12	
4	7.36 ± 0.20		8.38 ± 0.23	
7	7.89 ± 0.18	7.30 ± 0.15	8.47 ± 0.12	8.50 ± 0.17
10		7.45 ± 0.16		9.10 ± 0.18
11	7.62 ± 0.27		⩾ 10.54	
14		7.50 ± 0.27		⩵ 9.50

Table 2.7

Growth of cuttings of Telephone and Greenfeast peas. July/August. Natural SD (about 10 hours) Results and Standard errors.

Age of plant when cuttings taken (days)	F		Cb	
	Greenfeast	Telephone	Greenfeast	Telephone
7	17.00 ± 0.27	25.15 ± 0.90	15.25 ± 0.25	16.54 ± 0.24
10	17.29 ± 0.24		15.71 ± 0.17	
11		25.63 ± 0.79		18.25 ± 0.59
14	17.58 ± 0.23		15.58 ± 0.19	
17		25.47 ± 0.76		16.73 ± 0.56
18	20.86 ± 0.21		17.14 ± 0.29	
22	20.27 ± 0.55		15.00 ± 0.52	
27	17.61 ± 0.24		12.69 ± 0.69	
Control	20.73 ± 0.36	23.92 ± 0.55	14.81 ± 0.44	15.20 ± 0.43

Table 2.8

Comparison of first (Cb) and stable (Cbs) nodes of four-leaflet leaves in Greenfeast cuttings.

Age of plants when cuttings taken	Cb	Cbs	% R
7	15.25	15.25	0.00
10	15.71	15.71	5.88
14	15.58	15.58	0.00
18	17.14	17.43	14.30
22	15.00	16.00	45.00
27	12.69	15.31	77.00
Controls	14.81	15.27	27.27

% Reversion includes sequences of 4-3-4, 4-2-4, 3-2-4 and 3-2-3 leaflet/node.

Table 2.9

Growth of cotyledonary axillaries of Greenfeast and Telephone. Natural SD for Greenfeast (about 10 hrs.) 8 hrs. for Telephone. Results and Standard errors.

Age of plant when cuttings taken (days)	F		Ca	
	Greenfeast	Telephone	Greenfeast	Telephone
2		20.25 ± 0.99		11.92 ± 0.30
4		19.64 ± 0.71		12.86 ± 0.43
7	17.91 ± 0.64	20.33 ± 0.93	13.00 ± 0.71	12.87 ± 0.22
10	18.00 ± 0.32		13.31 ± 0.51	
11		20.60 ± 0.43		13.00 ± 0.32
14	17.14 ± 0.26		13.57 ± 0.20	
18	16.50 ± 0.40		14.66 ± 0.33	

Table 2.10

Growth of cotyledonary axillaries of Massey peas. Natural LD (about 16 hours).

Treatment	Time of decapitation (Days after germination)	F	n
+ cots + roots	4	6.90	10
	6	7.55	11
	8	7.38	13
	10	6.50	2
	12	7.00	2
- cots + roots	4	7.43	7
	6	8.00	6
	8	8.67	3
	10	-	0
	12	-	0
- cots - roots	4	-	0
	6	9.00	3
	8	8.50	4
	10	7.00	2
	12	-	0

Table 2.11

Growth of Greenfeast axillaries without cotyledons. Natural LD (about 16 hours).

Time of decapitation (Days after germination)	F	Ca	n
4	13.78	11.89	9
6	14.08	11.46	13
8	14.17	11.50	6
10	12.50	11.50	2
12	-	-	0

Table 2.12

Comparison of Ca and Cb in Greenfeast axillary cuttings. Natural LD (about 16 hrs.).

Time of decapitation (Days after germination)	Ca	Cb	n
4	11.50	11.50	2
6	12.00	12.50	10
8	10.00	$\geqslant$10.69	16
10	9.67	$\geqslant$11.00	6
12	-	-	0

Table 2.13

Length of shoot of Greenfeast axillaries grown under different conditions.

Natural LD (about 16 hrs).

Treatment	Time of decapitation (Days after germination)	L	n
+ cots + roots	4	27.00	15
	6	24.94	31
	8	25.14	22
	10	24.00	16
	12	22.14	7
- cots + roots	4	14.22	9
	6	21.38	13
	8	19.50	6
	10	-	0
	12	-	0
- cots - roots	4	14.00	2
	6	12.10	10
	8	10.31	16
	10	9.17	6
	12	-	0

iv. Experiments on Flowering and Vegetative Growth in "Embryo" Cultures.

Many workers have used the technique of "embryo" culture (i.e. culture of plants without cotyledons) to investigate the problems of growth in peas. Most of these have attempted to replace the effect of cotyledons by a complex system of chemical substances. One of the most detailed studies is that of Fries (1954), who found that many substances, in particular amino acids and vitamins, gave improved vegetative growth. His main measurements were on shoot height and number of lateral roots. Saubert von Hausen (1948) found that flowering only occurred in cultures when ascorbic acid was present. Similar results have been achieved with ascorbic acid on Trigonella and Brassica (Chinoy et al. 1957). Kruyt (1952) studied the effects of auxin-type substances on pea "embryos" grown in the dark. Gentcheff and Gustaffson (1940) found that IAA was necessary for development of pea flowers in culture. All these workers grew their plants in the culture medium throughout their experiments, which were thus of a rather short duration. Haupt (1952, 1954, 1957 etc.) scored his "embryos" for node of first flower and his measurements of vegetative growth were considered in the light of their secondary effects on flowering. Cruickshank (unpub.) in this laboratory has repeated some of Haupt's experiments and in particular confirmed the delaying effect of yeast extract on flowering. These experiments were made using early varieties. Several extensive experiments were made during the present project using both early and late varieties and these will be summarised here. The "embryos" were grown in sterile culture on agar for about two weeks, after which they were transplanted into soil in the glasshouse. A similar technique was used by Louis and Schopfer (1955) for "embryonic" pea grafts. Many of the "embryos" produced strong plants which bore one or more pods with viable seed.

Since many of the experiments with late varieties gave negative results, full details will not be given. In all cases the medium of Fries (1954) was used as a base, and was modified as required. Seeds were sterilised and soaked for about two hours before "embryos" were excised. Where many cultures were set up, some seeds

had a longer soaking time than others (2-8 hrs.). No effect of soaking time on vegetative growth or flowering node was observed. Cultures were placed in a constant temperature room (about 22°C) usually in continuous light (from fluorescent strips and incandescent globes).

Table 2.14 gives some of Cruickshank's preliminary data with Massey embryos. Cultures grown on modified Haupt's medium gave much poorer growth than those on the unmodified medium. The control cultures on both media flowered at similar nodes in spite of this difference in vegetative growth. All substances tested, except riboflavin, caused a significant delay in node of first flower. An interesting point is that extracts of both a late (Telephone) and an early variety (Massey) caused a flowering delay. This has been repeated using diffusates from the varieties Massey and Greenfeast, but another experiment showed no effect of diffusates (see Table 2.15). Using a whole, unattached cotyledon in each culture the results were variable but the number of survivors was low. All these results show that under some circumstances cotyledonary substance from early and late varieties can delay flowering of either type of variety. This is in contrast to unconfirmed work of Highkin (1955) who found that diffusates promote flowering. The action of the cotyledons is probably through amino acids or other organic substances such as those found by Haupt to delay flowering. A hormonal explanation seems unlikely because the effects of early and late varieties is similar. In all experiments height and often weight of the plants was also measured. Neither of these showed any significant difference between controls and diffusate treated embryos.

Several experiments have been made with various substances, none of which had an effect (in the concentration used) on flowering or vegetative growth of Greenfeast embryos. These substances were (w/v) Benzimidazole 10^{-4}, Adenine sulphate 10^{-4}, Adenosine 10^{-3}, Guanosine 10^{-3}, Guanine 10^{-3} and Hypoxanthine 10^{-3}. All these substances have been found to effect growth in some plants. In a few preliminary cultures with Gibberellic Acid, all the treated plants died. It appears that a dose

of 1µg can be toxic to pea embryos. The pea apex is probably highly sensitive to GA as other experiments have indicated (chapter 4).

Table 2.14

Effect of different media on node of first flower in Massey embryos

Data of Cruickshank (unpub.).

Medium	F	n
Haupt	9.30 ± 0.25	10
Modified Haupt. Control for 4 treatments below	9.67 ± 0.43	3
Modified Haupt + Massey extract (water sol.)	11.40 ± 0.57	5
Modified Haupt + Massey extract (ether insol.)	12.00 ± .000	4
Mod. Haupt + Telephone extract (water sol.)	11.75 ± 0.75	4
Mod. Haupt + Telephone extract (ether insol.)	12.50 ± 0.50	2
Mod. Haupt Control for 2 treatments below	10.67 ± 1.11	3
Mod. Haupt + riboflavin	10.63 ± 0.28	5
Mod. Haupt + yeast extract	13.00 ± 0.39	5

Table 2.15

Effect of cotyledons and their diffusates on growth of Massey and Greenfeast embryos. Node of first flower. Data for two diffusate experiments included.

Embryo	Cot. or diffusate	Diffusate				1 loose cotyledon	
		F	n	F	n	F	n
M	0	9.0 ± .32	5	9.67	6	10.6 ± .40	5
M	M	9.2 ± .14	6	9.57	7		
M	G	10.1 ± .26	7	9.86	14	12.0 ± 1.0	2
G	0	14.9 ± .35	8	12.17	6	13.0 ± .19	8
G	M	15.6 ± .24	5	12.51	12		
G	G	15.0 ± .00	2	12.50	12	11.5 ± .50	4

x. Indifference of Plants to Defoliation except under Short Days

Various workers have postulated that pea leaves play a definite part (other than as photosynethic organs) in the development and growth of this plant. Went (1938) suggested that mature leaves synthesise a leaf growth substance in the light. Paton (1956) found a quantitative relationship between the leaf area exposed to radiation and the node at which flowers are initiated in the late variety Greenfeast. According to the daylength, leaves of late varieties may produce colysanthin or flower hormone. Paton (1956) has postulated that the colysanthin from the cotyledons passes into the first two leaves where it is converted into flower hormone in long days. If these hypotheses are correct, it would be expected that defoliation should markedly alter both the flowering and the vegetative growth patterns of late pea varieties. Several defoliation experiments have been performed and in general they show that Greenfeast peas are remarkably indifferent to such treatments, especially in long days. For this reason, detailed results have not been given.

Experiment 2.4 Greenfeast peas were germinated as usual, and planted into boxes. The following defoliation treatments were made, stipules being left intact throughout.

1. First two true leaves (nodes 3 and 4) removed when young. (less than 1 cm. long and clasping apex).
2. Second two true leaves (nodes 5 and 6) removed when young.
3. First three young leaves removed.
4. First two leaves removed when fully expanded.
5. Cotyledons removed.
6. Control. Whole plants.

The experiment was carried out under natural short days (about 10 hours) and plants were scored for number of leaflets per node and F.

Figures for node of established four leaflet leaf (Cbs) and F when young leaves were removed are shown in figure 2.6. The linear regression line for stable node of four leaflet leaf on number of leaves removed was significant at the 0.1% level, the overall treatment effect only reaching the 5.0% level. The remaining data are shown in table 2.16.

Experiment 2.5 Greenfeast peas were grown as in experiment 2.4. Leaves were removed singly at each node from 3-8, both young and expanded. This resulted in 12 treatments. In addition there were three treatments in which the first 2, 4, and 6 leaves were removed. These, together with controls gave a total of 16 treatments. A large number of plants was grown, there being 20-30 survivors in most treatments. In this experiment natural daylight was supplemented to give an 18 hour light period. Plants were scored as in experiment 2.4.

There was no significant effect of treatment on F. Most treatments reduced the node of first four leaflet leaf, while not affecting the first node with more than two leaflets.

Experiment 2.6 In this experiment plants had either young or expanded leaves removed at the following groups of nodes:- 3 and 4; 5 and 6; 7 and 8; 9 and 10; 3, 4, 5 and 6; 7, 8 9 and 10; 3, 4, 5, 6, 7, 8, 9 and 10. Control plants were also grown. Plants were subdivided into two photoperiodic treatments in which they were given either an 8 or a 16 hour day.

Few significant effects of treatment were observed.

Discussion. The biggest treatment effect on F was obtained when the cotyledons were removed. This has been discussed earlier in the chapter. The only other significant delay in F was obtained in experiment 2.4 when young leaves at nodes 5 and 6 were removed. This is the only result that directly fits Paton's theory of the role of leaves in the flowering process. In long days about 6 leaves would have been expanded when flower initiation occured. Even when all these leaves were removed (i.e. about half the total photosynethetic area, since stipules were left intact) no effect on flowering was observed. This suggests that the correlation between leaf area and F may be one of chance, in that environmental conditions affect the two processes proportionally. It has been found in some experiments to be described in chapter 3, that small and large plants may have the same value of F, even when the leaf areas of the two are vastly different. On the other hand it is possible that defoliation may cause two equal and opposite effects on the plant e.g. a) reduction in rate of node formation tending to lower F, and b) reduction of photosynthetic area, which on Paton's theory should result in higher F.

Unfortunatoly, the number of expanded nodes was not measured accurately. If flowering node is a function of leaf area exposed x time (Paton 1956) then a reduction in the rate of node formation may increase the time of exposure of any leaf prior to flower initiation (if F remains constant). To explain the results of the severe treatments described in this section, a very great effect of treatment on rate of node formation would be necessary. This was not observed. Leaf removal does not necessarily reduced rate of node formation. Davidson and Donald (1958) found that defoliation could decrease or increase leaf production according to the growth of the plant (clover).

In experiment 2.4, there was a significant delay in four leaflet leaf production when some young leaves above node 4 were removed. Until 4 nodes are expanded, the seedling is partly or wholly dependant on its cotyledons for growth substances (see section ii) and this may be why removal of leaves at nodes 3 and 4 only has an effect when other leaves are also removed. In this experiment there is a quantitative relationship between number of young leaves removed and the delay in production of leaves with more than 2 leaflets. This confirms Went's (1938) suggestion that leaf growth substances are produced by leaves in light.

In long days only the extreme treatments (e.g. when 8 leaves were removed) had a significant effect in delaying production of four leaflet leaves.

The general evidence of the present group of experiments is that defoliation, under short day conditions especially, leads to a higher value of Ca. This indicates that leaf growth substances (i.e. lobing factors) are produced by the leaves, since a purely nutritional effect would be expected to result in smaller, rather than fewer leaflets. These results are in agreement with those of Hjoku (1956b) who found that defoliation reduced lobing of subsequent leaves. Whether or not these results are typical of all pea varieties is not known. Rignelli (1950) found that defoliation effects on lucerne were different with different varieties.

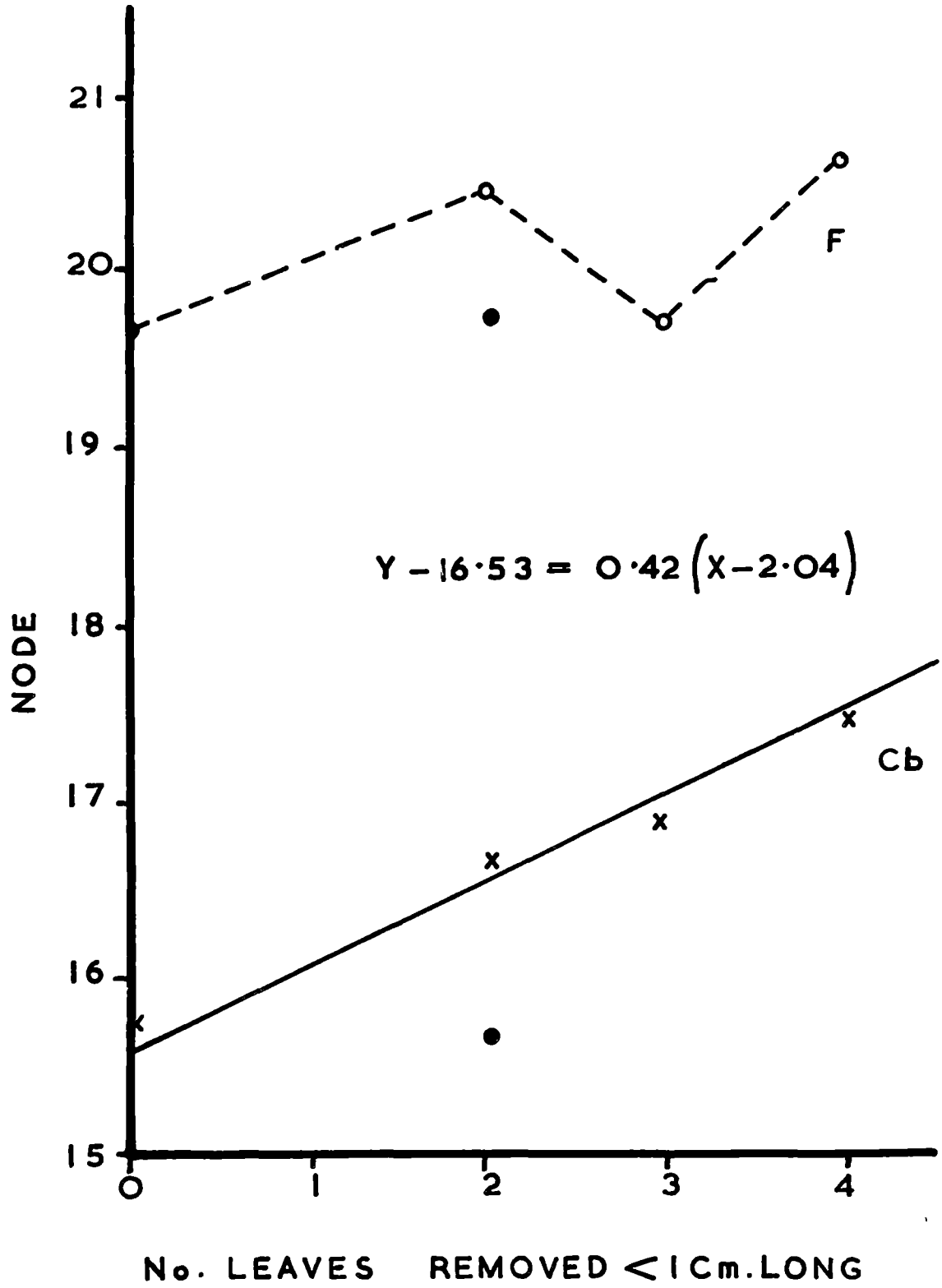


Fig. 2.6. Effect of removal of young leaves of Greenfeast peas on node of first flower (F) and first node with four leaflets (Cb). Solid circles are for leaves removed at nodes 3 and 4. Otherwise 2, 3 and 4 leaves removed at nodes 5 and 6; 4, 5and 6; and 3, 4, 5, and 6 respectively.

Table 2.16

Effect of defoliation on growth of Greenfeast peas. Natural SD (about 10 hrs.). Treatment means and their standard errors.

Treatment (leaves removed)	Ca	% R	F
1st 2 Young	14.82 ± 0.55	0.00	19.73 ± 0.47
2nd 2 Young	15.11 ± 0.59	33.33	20.44 ± 0.27
1st 3 Young	15.50 ± 0.77	20.00	19.70 ± 0.21
1st 4 Young	15.91 ± 0.41	36.36	20.64 ± 0.24
1st 2 Mature	14.64 ± 0.64	27.27	19.36 ± 0.28
Cotyledons	15.75 ± 0.14	0.00	17.58 ± 0.24
none	14.40 ± 0.51	33.33	19.66 ± 0.25

vi. Leaflet Number as a Measure of Leaflet Area

In many experiments in this department, leaflet number has been scored as an estimate of leaf growth and area. It seemed advisable to check the validity of this assumption, especially since Njoku (1956b) found that leaf area and lobing (= leaflet number) were not always correlated in Impatiens. These measurements were made before the photoelectric system was devised and therefore a planimeter was used.

Some typical results are given in figure 2.7. The minor fluctuations are probably due to environmental changes. The trends are essentially similar in long days, but the values for individual plants vary widely. Within each plant there seemed to be a maximum total area for the first two leaflets per node, after which any increase in leaf area was expressed as the formation of new leaflets. The maximum area for two leaflets is not the same for different plants.

In about one third of Greenfeast peas three or four leaflets are present at node four. No reason has yet been found for this behaviour. Where this occurs, the three or four leaflets at node four usually have a smaller area than the two at node 5.

It appears from these measurements, that in whole plants growing normally i.e. without chemical substances added or parts removed, leaflet number is a valid measure of leaf area. Under these conditions the two variables are highly correlated. It is suggested in the results presented in this thesis that treatments causing an abrupt change in the rate of node formation may separate factors governing the two processes (i.e. leaf area production and leaflet number production) (see chapter 3).

Leaflet number has been measured in most of the experiments described, because even where it is not a good indication of leaf area, it still gives a measure of "physiological age".

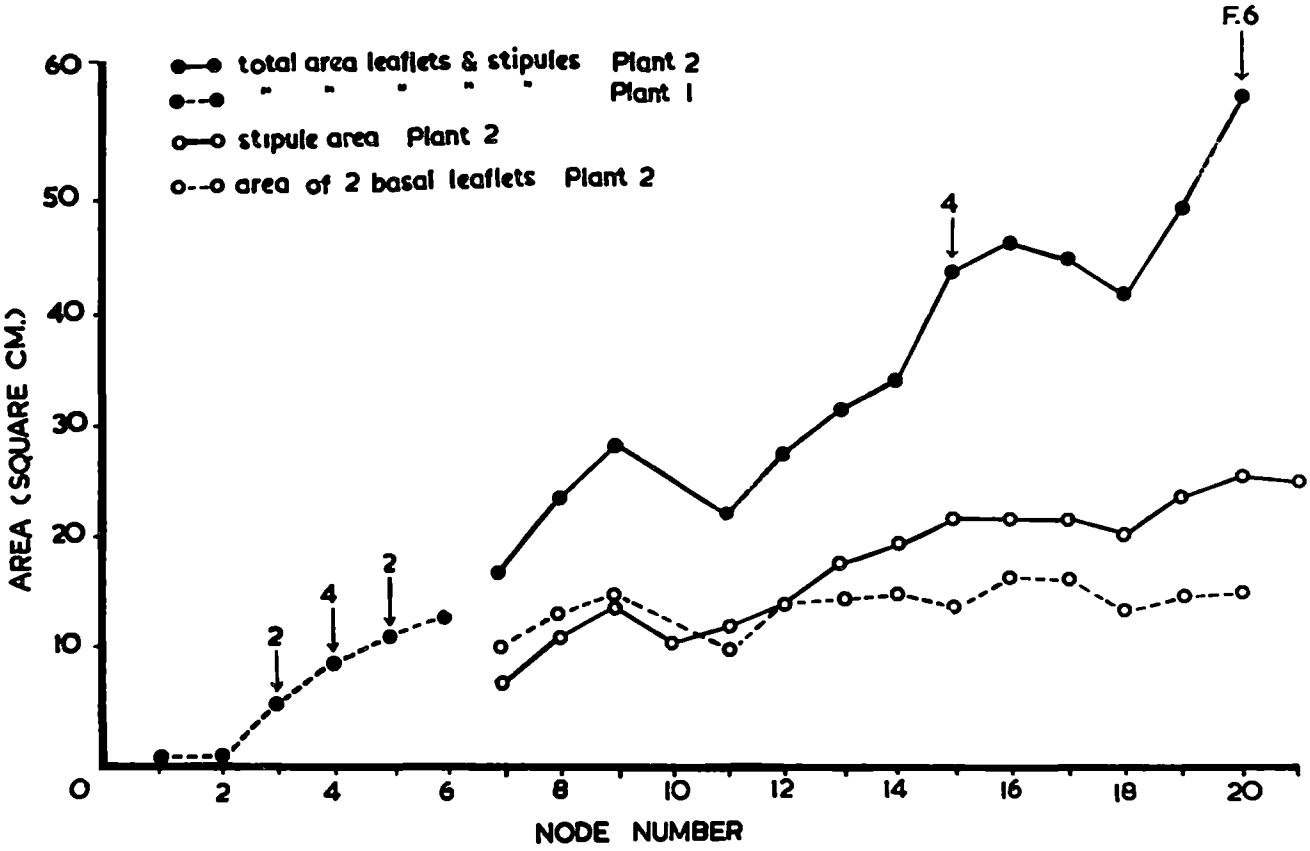


Fig. 2.7. Typical leaf area curves. Numbers and arrows indicate the number of leaflets at that node, when different from 2. F. indicates flowering node.

CHAPTER 3.

EFFECTS OF CHEMICAL SUBSTANCES AND LEACHING IN WATER ON VEGETATIVE AND REPRODUCTIVE GROWTH

i. Introduction

A general discussion of the effects of adenine on plant growth, and possible mechanisms for its action has already been given in chapter 1. In section ii. of this chapter, the effect of adenine in promoting general vegetative growth of peas will be described.

In addition to adenine, a number of other chemical substances which are known to affect growth of some plants was tested (section iii). In order to do this, the bases of the cuttings were immersed in a solution of the substance under test. This method of chemical treatment is very simple, and was used successfully by Paton (1956) for applying naphthalene acetic acid to pea cuttings. During the course of this work an interesting effect was noticed, in that leaching in water reduced the node number of first flower. A short note has been published describing this effect (Sprent and Barber 1957). The phenomenon was investigated further and the results are described in section iv. Here the effect is correlated with time and duration of treatment and various aspects of vegetative growth. The last section of this chapter deals with the effects of vernalization and daylength on the response to leaching.

ii. Adenine as a Stimulator of Vegetative Growth

The usual method of applying adenine to intact plants has been by spraying (Kruyt and Veldstra 1947) or through nutrient solutions (Bonner and Bonner 1940). Various other methods have been tried in the present work. These have been briefly mentioned in chapter 1, section ii. The only method to yield a significant effect of adenine was when the solution was applied to the cut base of the plumule. To do this, a piece of "polythene" tubing was selected to fit tightly round the cut stump, and a measured amount of solution was placed inside the tubing and in contact with the cut surface. The main results for this group are given in table 3.1. The data for shoot weight showed a significant treatment effect, but this could not be further localized by sub-division into leaflet + stipule weight (L + S) and petiole + tendril weight (P + T). These results will be discussed together with those for experiment 3.1 in which the plants were grown in liquid culture.

Experiment 3.1. Massey peas were germinated in the usual way. After 4 days the cotyledons were removed and the plants were supported by cork discs floating on a litre of culture solution in a beaker. Initially there were five plants grown in each beaker and three beakers in each treatment. At the time of transfer the plumules were 0.5 cm. long and no lateral roots had formed.

The basic culture medium was

Potassium nitrate	KNO_3	0.25g
Calcium nitrate	$Ca_3(PO_4)_2$	0.25g
Calcium sulphate	$CaSO_4 \cdot 2H_2O$	0.25g
Magnesium sulphate	$MgSO_4$	0.25g
Ferric chloride solution	5%	3 drops
Manganese chloride	$MnCl_2 \cdot 4H_2O$	traces
Distilled water		1000ml.
Trace element solution		1ml.

This was modified as follows, to give four treatments

(1) Control. Basic medium alone
(2) Basic medium plus 1mg/l adenine sulphate
(3) Basic medium plus 5mg/l adenine sulphate
(4) Basic medium plus 20mg/l adenine sulphate

Adenine sulphate was used in preference to the free base because of its greater solubility. Culture solutions were changed twice weekly and aerated throughout by means of a modified filter pump (kindly suggested by Dr. E. Scott, Biophysics Department). When the plants were a week old they were innoculated with a virulent strain of Rhizobium leguminosarum and the beakers were surrounded by black paper so that nodules could form. When the plants were 35 days old they were scored, owing to the onset of a bacterial infection. Plants were scored for F, Ca, degree of nodulation of roots, plumule weight and root weight.

Table 3.2 gives the average weights of plumule and of root. There is an optimum value for plumule weight at 5 mg/l adenine. The analysis of variance for this and for root weight yielded no significant treatment effect, because of the high variances. A "t" test between controls and 5mg/l gave a highly significant result, but as the variances were very different this may not be strictly valid. No correlation could be found between root weight and amount of nodulation. The difference between node of first flower for treated and untreated plants was significant at the 5% level. In no treatment did the plants form more than two leaflets per node.

Discussion Both this experiment and the preliminary one show that adenine can cause a general stimulation of vegetative growth as measured by fresh weight. This confirms the report of Went (1957 pp. 316-7) based on data of Galston with Alaska peas. He found that the effect of adenine was least in the region of optimum night temperature for stem and leaf weights. The night temperature in the present experiments would have probably tended to minimise the effect of adenine. The optimum adenine concentrations in the two experiments described here were different (1mg/l and 5mg/l) and both were lower than the concentrations reported by Went (up to 100mg/l using sprays). However it is difficult to estimate the actual amount of substance received by the plants, except where adenine was applied to the cut base of the plumule. There appears no doubt that optimum concentrations do exist, although these probably vary according to environment. No particularly large stimulation of leaf growth was observed in Massey, though it was in Alaska (Went 1957). The increase in weight in the plants treated with adenine was spread over all parts of the shoot. No effect was observed on the roots, in contrast to the figures quoted by Went and also those of Fries (1954) using "embryo" cultures.

These results indicate that adenine is probably involved in a fundamental aspect of growth common to all plant tissues, rather than acting as a specific organ forming substance. This supports the interpretation of Skoog and Miller (1957), who suggest that adenine acts as a precursor of kinetin.

Table 3.1

Effect on adenine on growth of cotyledonary axillaries of Massey peas when applied to the cut base of the plumule. Each plant received several applications of 0.1 ml. Plants grown in natural long days (about 16 hours).

Means and standard errors (in cases involving a significant difference)

Solution applied	Wt. L+S mg.	Wt. R+T mg.	Wt. Stem mg.	Total shoot wt. mg.	L.cm.	n
none	1422	375	1117 ± 536	2915 ± 494	20.11	9
Water	1148	301	1136 ± 194	2584 ± 410	18.70	7
1mg/l adenine	1778	484	1702 ± 183	4079 ± 363	23.04	11-12
5mg/l adenine	1356	319	1126 ± 128	2684 ± 355	20.04	12

Wt. of stem obtained from Total shoot wt. - wt. (R + T) - wt. (L + S).

Table 3.2

Effect of adenine on F_1 plumule and root weight of Massey peas grown in liquid culture.

Adenine sulphate mg/l	Plumule Wt. (mg).	Root Wt. (mg).	F	n
0	451.54	316.09	10.73	11
1	514.31	329.15	10.08	13
5	600.16	296.89	10.17	6-9
20	492.00	264.00	10.18	11

5mg/l adenine sulphate caused a significant increase in plumule weight. All adenine treated plants flowered significantly lower (5% level) than controls.

iii. Testing of a Variety of Chemical Substances for Possible Promoting Effects on Pea Growth

Many substances have been cited in the literature as having an effect on plant growth. Some of these were selected for testing on pea cuttings. Most of the substances used were nucleic acid components. Adenine was used in combination with guanine, and for comparison cytosine and uracil were included. Because of their possible role in flowering, (especially guanosine, Donner 1957) purine ribosides were also used. Sugar was used as a general metabolite and yeast extract as a source of growth substances.

Experiment 3.2 Cuttings of Greenfeast peas were taken when one internode was expanded (early) and when 3-4 internodes were expanded (late). They were soaked for 5 or 10 days in solutions of the chemical substances in the following proportions:

1. Control unsoaked.
2. Control water soaked
3. 1% sucrose
4. Adenine (A) + guanine (G) 50mg/l of each
5. Cytosine (C) + uracil (U) 50mg/l of each
6. A + G + sucrose
7. guanosine + adenosine
8. A + G + C + U + sucrose
9. Yeast extract

The treatments were combined factorially, the unsoaked cuttings being duplicated to keep the experiment orthogonal (because the combination of unsoaked with 5 and 10 day soaking was impossible). This resulted in 36 treatments, 9 chemical, with two cutting ages and two times of soaking. After soaking, the cuttings were planted into boxes in units of 5, the boxes arranged in 4 randomised blocks. Natural light was supplemented to give a 16 hour day. Plants were scored for height, flowering node, number of leaflets per node and number of expanded nodes.

The results are summarised in table 3.3. The plants which flowered at the highest node of all were the late unsoaked cuttings. All the late soaked cuttings flowered at a lower node, and in most cases, the longer the soaking time, the greater the reduction in node number of first flower. This effect was not observed in the early cuttings. The node of first four leaflet leaf was very uniform throughout all treatments, including the early and late cuttings.

Shoot height was consistently greater and more variable in the late cuttings. Most of the substances used caused a slight reduction in height. Similarly in the late cuttings most chemicals reduced slightly the number of expanded nodes. The late unsoaked cuttings grew best.

Discussion. The plants in this experiment grew satisfactorily and none of them showed any toxicity symptoms as a result of the chemical treatment. On the contrary, the plants showed little response to chemicals applied in this way. The one treatment which was markedly out of the general range was the late unsealed group of cuttings.

The late unsealed controls flowered at a higher node than the corresponding early cuttings. This was to be expected from the inhibitor theory already outlined. However, the late sealed cuttings flowered at a similar node to the entire group of early cuttings. In view of the evidence already obtained in this department, the results seem best explained as the leaching out of colysanthin from the late cuttings. It is possible that the interruption of some of the food supplies when cuttings are taken could lead to a breakdown of colysanthin. However, many substances probably diffuse out of the cut base of a plant shoot for purely physical reasons, especially when the cuttings are immersed in water alone. This may be a similar process to that observed by Fries and Foramn (1951) from pea roots and by Frank (1954) from Arabidopsis and Xanthium roots. In addition, many substances will pass to the base as a wound reaction to stimulate the production of callus and roots. The flow of substances in the shoot will therefore tend to be basipetal and colysanthin could be carried with the stream. It is possible that chemicals dissolved in water would not be able to enter the plant to any extent against this gradient. This is not true for auxins (Paton 1956), but may account for the lack of reaction of the cuttings to the chemicals tested.

Table 3.3.

Growth of Greenfeast cuttings soaked in solutions of chemical substances.

Cutting type	Chemical Treatment	Soaking time(days)	F	Cb	L	E
Cuttings taken when 1 internode expanded	none	0	14.1	13.5	23.9	13.5
	water	5	13.9	13.7	20.4	12.5
	"	10	14.2	13.8	21.8	13.6
	Sucrose (S)	5	14.1	13.8	21.6	13.3
	"	10	13.9	13.7	25.1	13.7
	Adenine (A)+Guanine(G)	5	14.1	13.9	19.8	13.0
	" "	10	14.4	14.2	21.2	13.5
	Cytosine(C) + Uracil(U)	5	14.6	14.6	20.5	13.5
	" "	10	14.0	13.7	21.6	13.2
	Adenosine + Guanosine	5	14.2	14.3	24.5	13.2
	" "	10	14.3	14.0	24.4	13.6
	A + G + S	5	14.1	13.8	21.2	12.6
	"	10	14.2	13.8	23.0	12.9
	A + G + U + S	5	14.1	14.0	19.6	13.0
	"	10	13.9	14.0	21.9	13.4
	Yeast extract	5	14.2	13.8	20.5	12.5
	" "	10	14.1	13.9	17.7	12.3
Cuttings taken when 3 - 4 internodes expanded	none	0	15.8	14.5	37.9	16.0
	water	5	14.6	13.7	34.3	14.9
	"	10	14.2	14.1	30.6	14.9
	Sucrose	5	14.7	14.3	32.7	14.6
	"	10	14.2	13.8	26.6	14.0
	Adenine + Guanine	5	14.8	14.0	32.7	14.6
	" "	10	14.2	13.6	28.3	14.2
	Cytosine + Uracil	5	14.5	13.9	26.5	14.4
	" "	10	14.0	13.2	26.7	14.5
	Adenosine + Guanosine	5	14.8	14.2	31.9	15.6
	" "	10	13.8	13.6	26.2	14.4
	A + G + S	5	14.3	13.9	26.8	14.7
	"	10	14.3	13.7	26.6	14.0
	A + G + U + S	5	14.5	14.1	26.5	13.8
	"	10	14.6	14.0	25.0	14.3
	Yeast extract	5	14.9	14.3	30.1	14.4
	" "	10	14.7	14.0	27.3	14.8
Effect			significance levels			
Cutting type (CT)			0.1%		0.1%	
Soaking time (ST)			5.0%		n.s.	
Chemical treatment (C)			1.0%		1.0%	
C X ST			n.s.		n.s.	
C X CT			1.0%		n.s.	
CT X ST			n.s.		n.s.	
CT X ST X C			n.s.		n.s.	
L.S.D. 5%			0.6		7.8	
L.S.D. 1%			0.9		10.3	

ix. Leaching of a Flower Inhibitor from Greenfeast Cuttings.

A brief mention of the effect of leaching on flowering of Greenfeast cuttings was made in the previous section. This effect emphasises the labile nature of colysanthin and suggests that it may ultimately be chemically definable. In this section an experiment will be described in which the leaching phenomenon was further investigated.

Experiment 3.3 Cuttings of Greenfeast peas were taken at 6, 11, 15, and 20 days after germination and soaked in distilled water for 0, 4, 8, 12 or 16 days before planting into soil in boxes arranged in a randomised block design. The plants whose development at time of planting is given in table 3.4 were grown in natural summer photoperiod (about 16 hr.). Plants were scored for F, Ca, Obs, I, and on an arbitrary general growth scale (G) in which number of chlorotic leaves etc. were taken into account. Figures obtained for flowering have already been described (Sprent and Barber, 1957), but for reference they are summarised in figure 3.1 and Table 3.5.

Data for the other variables are given in tables 3.5 together with their analyses of variance. Several regression analyses were also made. In this way no correlation could be established between flowering and growth estimate, or between node of first four leaflet and flowering. There was a significant linear regression of shoot height on growth estimate. The deviations from linearity were significant at the 5% level. Between number of expanded nodes and cutting date, a quadratic line fitted better than a linear, one, but the turning point (maximum) is of doubtful biological significance. Linear regressions of node of first flower on leaching time for the different cutting dates are shown in figure 3.1. All the analyses are given in table 3.6.

Discussion The data for node of first flower are in accord with the theory that an inhibitor passes from the cotyledons to the shoot. It appears to remain in a labile state for some days during which time it is removable by leaching. At about fourteen days after germination the inhibitor becomes incorporated into the cytoplasm and a state of "ripeness to flower" is achieved. This time equals that found in experiment 2.1, when removal of cotyledons had no effect. Soon after this the actual initiation of flowers begins. In most experiments performed in this department on flowering in peas, it has been found difficult to exclude completely the hypothesis that flower inhibition may be a direct result of a faster growth rate. This possibility has been virtually excluded by the present results, in which no correlation could be established between vegetative growth measured as shoot height, general growth estimate or node of first four leaflet leaf, and node of first flower. It appears, therefore, that a true

flower inhibiting substance is responsible for at least a part of the effects noticed.

The relationships between the various methods of measuring vegetative growth are of interest. For most purposes shoot height seems to be a fairly good measure, as is shown by the close linear relation between this variable and the arbitrary growth scale. However the deviations from linearity are significant at the 5% level. This is most probably due to dying of the lower leaves in the cuttings taken at 20 days after germination. In these plants the heights may have been similar to those in other treatments but the number of surviving, functional leaves was less. In all cases except the 6 day cuttings, which were very small, soaking for four days seemed to promote vegetative growth. It is possible that water may have been absorbed to give larger cells and hence increased height. In addition, a few days in water may have promoted the growth of callus which is a necessary precursor of root formation.

The behaviour of cuttings leading to the formation of leaves with more than two leaflets is complex. In a previous experiment (2.2) some cutting treatments produced more than two leaflets at an earlier node than control cuttings. The present experiment may give more details of this phenomenon. By inspection of table 3.5 it can be seen that in general the treatments which tend to reduce growth also tend to reduce Ca. This is surprising because for nutritional reasons the relationship may be supposed to be an inverse one, if the number of leaflets is always an adequate measure of leaf area. During the course of the present investigation it has seemed that number of leaflets and leaf area may not always be inseparable. Njoku (1956) has postulated a leaf lobing substance to be formed in the leaves of Ipomoea, and it is not impossible that a similar substance is formed in peas. In this case "lobing" is complete and leads to the formation of leaflets.

If some of this "lobing" substance is present in the cotyledons and more is produced in the leaves in light, a steady supply will be available to the growing apex. When the supply is increased to a certain level, leaves with more than two leaflets can be formed. If growth were temporarily interrupted, there would be an accumulation of "lobing" substance in the apex until growth is resumed. This could lead

to production of one or more leaves with more than two leaflets at an earlier stage than usual. As growth is resumed these plants revert to the 2 leaflet leaf condition until enough leaves are formed to give a stabilised 4 leaflet leaf condition. The latter occurs at a lower node in the later cuttings where the shoots have been in contact with the cotyledons for a longer period.

This tentative hypothesis forms a useful working basis and seems to explain most of the results obtained to date.

A flower at a given node is determined in advance of the leaflets at that node. This is to be expected from considerations of leaf histogenesis. The results of this experiment (table 3.5) show that leaflet determination is at least 3 nodes behind flower determination. In the 20 day cuttings flowering at node 14 is established, whereas the leaflets at nodes 11-12 are not since leaching can reduce the node of stable four leaflet leaf.

Table 3.4

Investigation of leaching of flower inhibitor from Greenfeast. Development of cuttings at time of planting.

Cutting age	Leaching time	L	Plants rooted	No. roots	Root length cm.	E
6	0	1.0	0	0	0.0	2.0
6	4	1.5	0	0	0.0	3.0
6	8	1.5	20%	1	small	4.0
6	12	-	85%	2-6	1.5	-
6	16	1.5	96%	1-5	$\leq$ 2.0	4.0
11	0	3.0	0	0	0.0	3.5
11	4	-	0	0	0.0	-
11	8	-	92%	1-6	$\leq$ 1.0	-
11	12	4.0	100%	2-10	$\leq$ 4.0	5.0
11	16	4.0	100%	1-10	$\leq$ 4.0	5.5
15	0	3.5	0	0	0.0	4.5
15	4	-	0	0	0.0	-
15	8	-	100%	1-6	$\leq$ 5.0	6.0
15	12	5.0	100%	1-10	$\leq$ 4.0	5.5
15	16	-	100%	many	-	-
20	0	4.5	0	0	0.0	5.5
20	4	-	0	0	0.0	6.0
20	8	5.5	-	0-10	$\leq$ 5.0	5.5
20	12	-	100%	-	-	-
20	16	7.0	100%	many	$\leq$ 5.0	7.0

Table 3.5

Growth of Greenfeast cuttings of different ages soaked in water for different times.

Variable	Leaching Time (days)	Cutting date (days after germ.)				Significance Levels		
		6	11	15	20	Date	Time	D X T
F	0	14.3	14.5	13.6	14.4	0.1%	1.0%	1.0%
	4	14.0	13.4	14.0	14.2			
	8	13.9	13.3	13.6	14.0			
	12	13.6	13.0	13.1	14.3			
	16	12.8	12.4	13.3	14.1			
Ca	0	-	13.3	11.3	11.4	0.1%	5.0%	n.s.
	4	-	12.7	12.3	11.3			
	8	-	13.1	12.3	11.2			
	12	-	12.4	11.4	11.1			
	16	-	11.9	11.6	11.0			
Cbs	0	-	14.0	13.0	12.7	0.1%	0.1%	n.s.
	4	-	13.2	13.0	12.3			
	8	-	13.8	13.5	12.5			
	12	-	13.5	12.3	11.9			
	16	-	12.9	12.2	11.8			
L	0	7.8	13.4	11.5	10.8	0.1%	0.1%	0.1%
	4	6.2	15.4	14.2	13.4			
	8	6.1	13.4	13.1	12.1			
	12	6.3	10.8	11.6	10.1			
	16	4.8	9.8	8.0	8.9			
E	0	9.2	10.8	10.9	10.7	0.1%	0.1%	0.1%
	4	8.9	11.4	11.5	12.2			
	8	8.6	11.1	11.9	11.8			
	12	8.6	10.5	11.0	11.1			
	16	8.2	10.0	9.7	11.0			
G	0	2.8	5.1	4.1	3.0	0.1%	0.1%	1.0%
	4	2.3	5.5	5.5	3.7			
	8	1.8	4.6	4.5	3.6			
	12	2.0	3.6	3.0	1.9			
	16	1.4	3.2	0.8	0.8			

Table 3.6

Regression analyses of variance for F on leaching time.

Type of cuttings	Effect	Degrees of freedom	Mean square	Signif. level
6 day	Regression	1	4.41	0.1%
	Deviations	3	0.63	5.0%
	Error	20	0.17	
11 day	Regression	1	8.69	0.1%
	Deviations	3	1.09	n.s.
	Error	20	0.38	
15 day	Regression	1	0.56	10.0%
	Deviations	3	0.42	n.s.
	Error	20	0.15	
20 day	Regression	1	0.09	n.s.
	Deviations	3	0.11	n.s.
	Error	20	0.26	

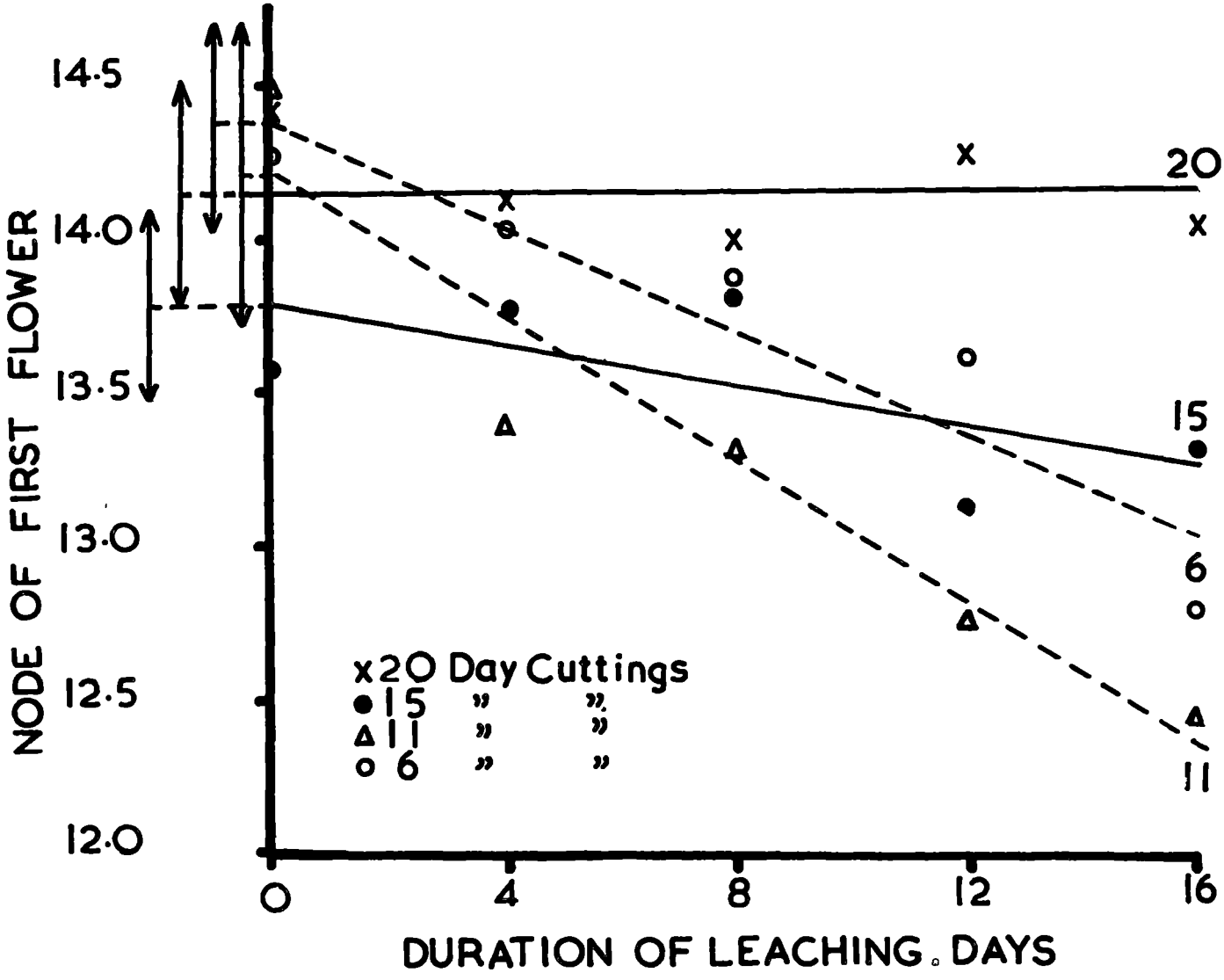


Fig. 3.1. Effect of leaching in water on node of first flower (F) in Greenfeast cuttings. Regression lines of F on leaching time for difference cutting dates. Arrows indicate the 95% confidence limits of the intercepts made by the lines on the y axis.

v. Interactions between Leaching Effect and Environment.

In section iv the optimum conditions for the leaching of colysanthin from cuttings were determined. As the experiment was carried out under long day conditions, it is clear that long photoperiods do not normally remove all colysanthin. Intact vernalized plants grown under long days seldom flower at a node lower than 14. This is largely true also for unsoaked cuttings, although many of the leached cuttings flowered at node 12. If such a large quantity of inhibitor could be removed under long days, it seemed possible that more could be removed under short days when it is not normally inactivated. Experiments 3.4 and 3.5 show that the magnitude of the leaching effect is similar under short and long days. They also show that even in vernalized cuttings grown under long days, leaching in water can still reduce the node of first flower.

If, as Paton (1956) suggests, colysanthin passes into the first two leaves to be inactivated, then removal of these leaves may reduce the leaching effect. If some of the colysanthin is also present in the stem, which seems almost certain since it must pass through the stem to reach the leaves, then cuttings taken above several internodes should show a smaller response to leaching than those taken just above the cotyledons. These possibilities were investigated in experiments 3.6 and 3.7 and shown not to be exactly true.

Experiment 3.4 In this experiment Massey was used in addition to Greenfeast to make sure that the leaching effect could not be obtained with early varieties. Seeds were germinated in the usual way, some in the glasshouse and some in the cold room (5 - 6°C). The latter were left in the cold for three weeks, and the unvernalized seeds planted so that when the cuttings were taken the two series were in a corresponding state of growth. Until the cuttings were taken, all plants were kept in short day (9 hours). They were then divided into the following treatments

early (2 nodes visible) and late (3 - 4 nodes visible) cuttings
short days and long days
vernalized and unvernalized (seedlings)
soaking times of 0, 12 and 18 days
early (Massey) and late (Greenfeast) varieties

The plants in short and long days were kept separate for practical reasons but within these limits the plants were grown in randomized blocks in units of 6 (long days) or 8 (short days). The long day plants had the natural winter day-length supplemented to 18 hours with Mazda horticultural lamps.

The results of this experiment were very disappointing as a large number of plants died due to poor conditions. No disease was apparent. Insufficient Massey plants survived for any conclusions to be drawn. The Greenfeast flowering data is summarised in Table 3.7. No factorial analyses were made as the data were too far from orthogonality. A single classification analysis of variance was made of first flower and shoot height, but no significant treatment effects were observed within the daylength treatments. This was due to the small numbers of survivors.

Experiment 3.5 Cuttings were taken from excess plants from the experiment 4.2. The four varieties Massey, Alaska, Greenfeast and Telephone were used, both vernalised and unvernalized. The treatments had to be restricted according to the number of plants and greenhouse space available. Since the varieties Massey and Alaska are non-photoperiodic and non-vernalizable, cuttings were taken only from unvernalised stock and grown in long days. The Greenfeast and Telephone plants were subdivided into vernalized and unvernalized, each under either long or short days. For the latter, natural short days (about 10 hours) were used. Long day treated plants (18 hours, given with supplementary light) were grown in another glasshouse, where the light could not affect the short day treatments. 20 plants of each treatment were grown, subdivided into units of 10 in randomised blocks. The cuttings were taken when about 3.5 nodes were expanded, which was expected to give maximum leaching effect, and the soaking times were 0 and 14 days.

Plants were scored for node of F, Ca, U and $l_{g.7}$.

The figures for Massey and Alaska are summarised in table 3.8. It can be seen that leaching had no effect on flowering node and little on other variables.

The data for Greenfeast and Telephone are summarised in table 3.9. Because the data is non-orthogonal, normal methods of analysis could not be used. Where the results were of special interest they were analysed by the method of fitted constants or by weighted squares of means according to the absence or presence of significant interactions (table 3.10). (The procedure followed was that outlined by Goulden 1952).

Experiment 3.6 Seedlings were germinated as usual. Cuttings were subsequently grown in soil in boxes arranged in randomised blocks. Natural daylight was supplemented to give a daylength of 16 hours.

The treatments are described in the table overleaf, together with the abbreviations used in the tables and graphs of the results. This set of treatments was duplicated, one half being leached (prefixed L) and the other being control (prefixed O). Treatments 1-6 were all made at the same time and thus treatment 1 serves as a control for treatment 6. Treatments 7 and 8 were made when 2 expanded leaves were present on the apical cuttings. The leached plants were soaked in water for 14 days prior to planting.

Treatment Number	Abbreviation	Description of treatment
1	C	Control cuttings.
2	34X	First 2 leaves (Nodes 3 and 4) removed while immature - prior to taking of cuttings.
3	34S	First 2 leaves removed when expanded - at same time as cuttings taken.
4	56X	As 2 but second pair of leaves removed (nodes 5 and 6).
5	56S	As 3 but second pair of leaves removed.
6	A2	Cuttings taken above node 2.
7	A4	Cuttings taken above node 4.
8	A6	Cuttings taken above node 6.
9	AC4	Control cuttings (entire) taken at the same time as A4 cuttings.
10	AC6	Control cuttings corresponding to A6.

Plants were scored for F, Ca, E, G, total length, and lengths from nodes C-2, 2-4, 4-6, and 6-8 as applicable. In scoring apical cuttings the nodes were numbered by adding on the number left on the stock i.e. the first node on plants from treatment A6 would be node 7. Thus all nodes were comparable.

Results The figures for apical cuttings for flowering, number of expanded nodes and general growth are shown graphically in figures 3.2,-3.4, together with the appropriate controls. All showed significant treatment effects. The least significant differences (L.S.D.) were calculated from the pooled estimate of error.

$$\text{L.S.D.} = t \times \sqrt{\text{error mean square} \times 2/R}$$

where "t" is the appropriate value of Student's "t" and R is the number of replicates.

All the length data are presented in table 3.11. Very few results are of interest. Table 3.12 shows the flowering, nodes expanded and growth data for defoliation treatments. No significant effect was observed. The data for Ca is not shown but follows the usual trend of lower values when cuttings are scaled.

Discussion There were insufficient plants surviving in experiment 3.4 for any definite conclusions to be drawn. However the data serves to confirm that obtained in experiments 3.3 and 3.5.

The survival rate in the vernalised Tolephone treatments in experiment 3.5 was very low. In all other treatments, however, a leaching effect is seen in which the node of first flower is reduced. This reduction is of the same order in all treatments, though possibly greater in long days. This suggests two alternative hypotheses. The first is that the inhibiting system is more complex than was at first thought. The second is that leaching only removes the inhibitor which is present in the cotyledons (and not any that has been manufactured by leaves in S.D.). This quantity would be the same in all plants, and thus the leaching effect could easily be the same in long and short days. In the absence of further evidence the second hypothesis seems the more likely. Leaching reduced the node of first flower in long days to the level of vernalised unsoaked cuttings. The flowering node of vernalised cuttings can be reduced by one node by leaching. It appears from these results that even leaching does not remove all of the salysanthin present in the cotyledons. It is possible that a longer period of leaching may remove more inhibitor, although it would almost certainly be detrimental to the growth of the cuttings. As the leaching effect on vernalised cuttings is less than that on unvernalised cuttings, it seems that the two processes (vernalization and leaching) act on the same system in the plant.

The effect of leaching on vegetative growth characters (U, Ca and L) is similar to that obtained in experiment 3.3. In the present case the longer soaking time (14 days) gave a similar promotion of vegetative growth to the 4 day treatment of the early experiment. The two varieties Greenfeast and Tolephone gave similar responses to leaching although the magnitude may be smaller in the latter.

The node of first flower in apical cuttings follows the same trends as in the data obtained by Paton (1956). The corresponding entire cuttings give similar values to those in experiment 2.2. The apical cuttings in general have a lower node of first flower than the entire cuttings, although this difference only reaches significance when the cuttings were taken after 4 nodes had fully expanded. This confirms

Paton's theory that the inhibitor passes from the cotyledons into the first two leaves. The results however, could be equally well explained by the inhibitor being confined to the first 4 internodes. This second alternative could explain the slight (but not significant) reduction in flowering when cuttings were taken above node two.

There were no significant differences between the leached apical and the leached entire cuttings. The overall trend for the two types is in complete agreement with the results given earlier in this chapter (section iv). When six nodes were expanded most of the plants had reached a state of "ripeness to flower". Cuttings taken at this stage show only a slight effect of leaching. If the leaves play a part in the flowering processes of peas, they must exert their effects before this stage! i.e. the leaves at nodes 3, 4,5 and 6 are the only ones which can be involved. These are the nodes which have been defoliated in this experiment. The results for flowering in the defoliation treatments are shown in table 3.12. It can be seen that all the leached cuttings flowered at the same node. All the unleached cuttings flowered about 1.5 nodes later. None of the defoliation treatments had any significant effect within these groups. This confirms the results given in experiment 2.4, that defoliation gives rise to little response in peas. It was at first thought that the lack of response to defoliation may be due to the larger effect of the cotyledons at this stage in the growth of the seedling. The present results cannot be explained in this way, since the cuttings were not in contact with the cotyledons. The defoliation treatments have been repeated yielding exactly similar results.

The results given in this thesis indicate that the colymanthin does not necessarily pass into the leaves, but may only be present in its labile state in the stem while being transported from the cotyledons to the apical bud. This is probably an over simplification, however, as it appears certain that the inhibitor can sometimes be counteracted in some way. Three experiments have been described in which Greenfeast cuttings have been taken at different intervals after germination. In one of these (experiment 2.2) the node of first flower showed a marked response to treatment. This response was easily explained on the inhibitor theory. The present

experiment showed a similar, though smaller, response. The results of experiment 3.3 showed no response of flowering to treatment in the unleached cuttings, agreeing with the results of Barber for Tolephone. Inhibitor was present in the cuttings of experiment 3.3 since it could be removed by leaching. In Greenfeast the response is usually greater in short photoperiods. It seems that in long days a second, unknown, factor comes into play making the effect of colysanthin in unleached cuttings. How far this second factor is involved in other experiments is not known.

A comparison of figures 3.2 and 3.3 shows that node of first flower and number of expanded nodes (E) are not highly correlated in this experiment. In all cases the value of E for leached cuttings is higher than that for unleached ones. This result seems to be fairly general, although the reason for it is not at the moment known. It would be interesting to see if it is related to a faster rate of root production in leached cuttings. The low values of E for apical cuttings taken above nodes 2 and 4 probably reflect a reduced capacity to resume growth. The increase seen in the figure for cuttings above node 6 is not a true treatment effect, since most of those nodes were expanded when the cuttings were taken. Even in this extreme treatment, leaching increased the value by one node. It seems most likely that water is the limiting factor for node expansion under these conditions. When the supply of water is plentiful the rate of node expansion is increased until some other factor, presumably nutritional, becomes limiting. This factor is unlikely to be carbohydrate since removal of leaves has no effect on number of expanded nodes (table 3.12). It may be mineral salts, nitrogen or other soil constituent.

A better estimate of the effects of treatment on general growth can be seen in figure 3.4, in which the arbitrary scale is similar to that used in section iv. Here there is a very close correlation between the severity of the treatment and growth. There is little difference between the leached and unleached cuttings. The differences between these curves and those for E are due to the fact that in the former the dying of some of the lower leaves in the more severe treatments is not shown.

Defoliation had little effect on either E or the general growth.(Table 3.12).

The length data (table 3.11) show that where soaking in water increases length the effect is usually located in the lower internodes. This would be expected if the effect was due to water supplies. Removal of leaves from cuttings caused a slight reduction in length. This is probably a nutritional effect.

Identification of extract. Since the effect of leaching on flowering node was discovered, numerous attempts have been made to concentrate the leachate. The concentrate has been tested on cuttings and whole plants of Massey and Greenfeast. Some of these tests were not considered satisfactory for various reasons. Some of them has shown a significant effect of the leachate in delaying the node of first flower, although most have shown a small delay. Further tests are being carried out. It may well be that colysanthin is not stable under the conditions of extraction.

During the course of the attempts at identification, some cuttings were leached while in a constant temperature room at 23°C in continuous light. A portion of these cuttings was enclosed in a dark chamber for the two weeks leaching time. Cuttings were then planted into the glasshouse. Surprisingly, the dark treated cuttings, though very chlorotic, survived in sufficient number (20) to be compared with the light treated cuttings. The latter showed a typical decrease in node number of first flower (13.90) compared with the unleached cuttings (14.80). The dark treated cuttings flowered at node 15.85. All cuttings had 2 expanded leaves, suggesting that these organs, being the major photoreceptors, may have a promoting effect on flowering. This promotion may simply be a photochemical destruction of colysanthin. The alternative that more colysanthin is synthesised by leaves in the dark seems unlikely in view of the results published by Tashima (1953) and Haupt (1957) using radishes and early peas respectively. These workers found that long days or darkness resulted in similar flowering nodes, but short days gave a significant delay. In view of the results obtained in this department, the data of Tashima and Haupt seem to indicate production of colysanthin in short days. Haupt's peas had cotyledons (and consequently

colysanthin or precursors) removed. The cuttings grown here were taken after a maximum amount of inhibitor had been transported from the cotyledons. It is not known whether this was removed by leaching in the dark or not. This point must remain unexplained until a method of testing leachates has been successfully worked out.

Dark treatment did not affect the cutting length, it increased the first node with more than 2 leaflets and decreased the number of expanded nodes.

Another interesting sideline arose from attempts to isolate the inhibitor. During the course of leaching large numbers of cuttings, the main apices of some died. These cuttings were grown and one axillary allowed to mature. In one series the dying main shoot was removed and in another it was left. It was found that the main apex did not affect flowering but it inhibited vegetative growth in the axillaries, which apparently could not use it as a source of food.

Table 3.7

Data from first experiment to test the effect of vernalization and photoperiod on the leaching of colysanthin from Greenfeast cuttings.

Vern.	Cutting type	Soaking time	daylength	F	n
UV	late	0 days	S.D.	24.67	6
UV	"	12	"	24.25	12
UV	"	18	"	24.77	13
V	"	0	"	no survivors	
V	"	12	"	17.50	2
V	"	18	"	20.33	3
UV	early	0	"	25.00	2
UV	"	12	"	24.43	14
UV	"	18	"	24.00	3
V	"	0	"	no survivors	
V	"	12	"	23.00	3
V	"	18	"	no survivors	
UV	late	0	L.D.	16.67	3
UV	"	12	"	15.00	7
UV	"	18	"	13.30	9
V	"	0	"	13.33	3
V	"	12	"	12.40	5
V	"	18	"	12.00	2
UV	early	0	"	16.00	2
UV	"	12	"	16.00	5
UV	"	18	"	12.00	1
V	"	0	"	no survivors	
V	"	12	"	13.67	3
V	"	18	"	no survivors	

Table 3.8

Effect of leaching on Massey and Alaska cuttings. Treatment means ($\bar{x}$) and number of plants per treatment (n).

Treatment	F		Ca		U		lg - 7cm.	
	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n
Alaska control	10.50	8	10.17	6	8.00	8	13.00	8
leached	10.43	7	10.00	7	9.43	7	14.00	7
Massey control	9.88	16	10.40	15	8.67	15	7.69	16
leached	10.11	9	10.14	7	7.33	9	7.71	7

Table 3.9

Effect of leaching on Telephone (T) and Greenfeast (G) cuttings. Treatment means.

Variety	Vern.	Photo-period	F	Ca	U	L 2-7cm.
T control	UV	L.D.	18.00	16.25	12.00	16.79
T leached	UV	L.D.	16.80	16.00	13.00	17.20
T control	V	L.D.	14.50	15.00	12.00	11.25
T leached	V	L.D.	13.00	-	-	13.50
T control	UV	S.D.	20.55	18.33	16.08	14.33
T leached	UV	S.D.	20.42	19.40	17.08	15.17
T control	V	S.D.	18.50	18.00	16.00	10.00
T leached	V	S.D.	17.75	17.00	16.25	12.50
G control	UV	L.D.	15.92	~14.36	9.50	5.33
G leached	UV	L.D.	13.64	12.57	10.50	5.71
G control	V	L.D.	13.36	12.10	8.73	4.73
G leached	V	L.D.	12.41	11.76	9.71	5.00
G control	UV	S.D.	20.09	16.82	14.83	4.83
G leached	UV	S.D.	19.00	16.67	14.81	5.25
G control	V	S.D.	16.29	14.75	12.47	4.18
G leached	V	S.D.	15.44	14.20	12.69	4.56

Table 3.10

Analyses performed on Telephone and Greenfeast cuttings for F.

Variety	Effect	ss	d.f.	m.s.	F	Sig.
T. I.D.	V	45.504	1	45.504	87.51	0.1%
	L	12.383	1	12.383	23.81	0.1%
	V X L	0.090	1	0.090	0.17	n.s.
	Error	7.800	15	0.520		
G. I.D.	V	35.062	1	35.062	120.82	0.1%
	L	48.181	1	48.181	166.03	0.1%
	V X L	5.920	1	5.920	20.39	0.1%
	Error	14.801	51	0.290		
G. S.D.	V	197.143	1	197.143	148.44	0.1%
	L	13.766	1	13.766	10.36	1.0%
	V X L	0.200	1	0.200	-	n.s.
	Error	74.376	56	1.328		

Table 3.11

Treatment means for total length (L) and a length of the first four pairs of internodes. All figures in cm.

Treatment	L	L_{0-2}	L_{2-4}	L_{4-6}	L_{6-8}
CC	18.15	1.48	1.68	2.10	4.15
L C	15.95	1.68	2.08	1.78	2.83
C12Y	12.10	1.43	1.53	1.45	3.05
I12Y	13.83	1.80	2.13	1.50	2.70
C12E	10.38	1.73	1.58	1.68	3.25
I12E	13.15	1.53	1.73	1.38	2.60
C22Y	14.05	1.20	1.58	2.00	3.53
I22Y	13.00	1.83	1.85	1.48	2.43
C22E	12.33	1.75	1.48	1.73	3.03
I22E	13.25	1.38	1.98	1.73	2.33
C12	10.45	-	1.20	1.93	3.43
I12	7.28	-	1.60	1.88	1.38
C14	4.75	-	-	1.25	2.03
I14	3.73	-	-	2.05	1.15
C16	3.60	-	-	-	1.43
I16	6.68	-	-	-	2.55
CAC4	13.08	1.35	2.05	1.90	2.60
LAC4	16.48	1.48	2.20	2.13	2.43
CAC6	11.38	1.25	2.23	3.03	2.10
LAC6	13.13	1.28	2.20	2.78	3.03

Table 3.12

Cuttings with leaves removed. Data for F, E and G.

Leaching	Age of lvs. removed	nodes defoliated	F	E	G
+	-	-	14.33	10.05	6.30
-	-	-	13.05	10.48	6.15
+	young	3, 4,	14.70	9.60	4.83
-	young	3, 4,	12.98	9.83	5.78
+	mature	3, 4,	14.40	9.63	5.35
-	mature	3, 4,	12.93	10.30	5.25
+	young	5, 6,	14.20	9.70	5.88
-	young	5, 6,	12.90	10.28	4.78
+	mature	5, 6,	14.43	9.58	5.05
-	mature	5, 6,	13.05	10.38	4.95
L.S.D. 5%			0.51	0.90	1.44
L.S.D. 1%			0.68	1.19	1.92

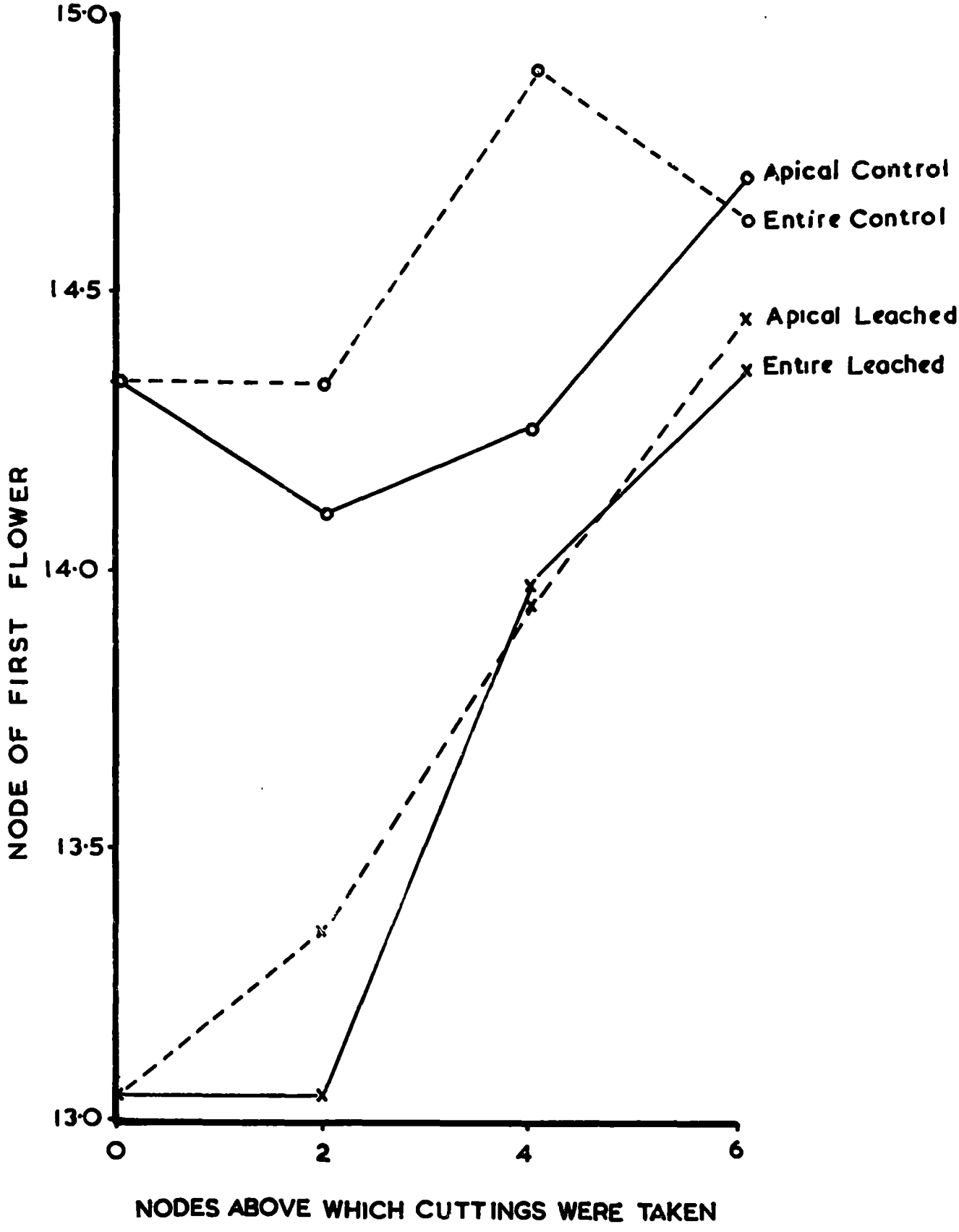


Fig. 3.2. Comparison of the effects of leaching on node of first flower for entire and apical Greenfeast cuttings.

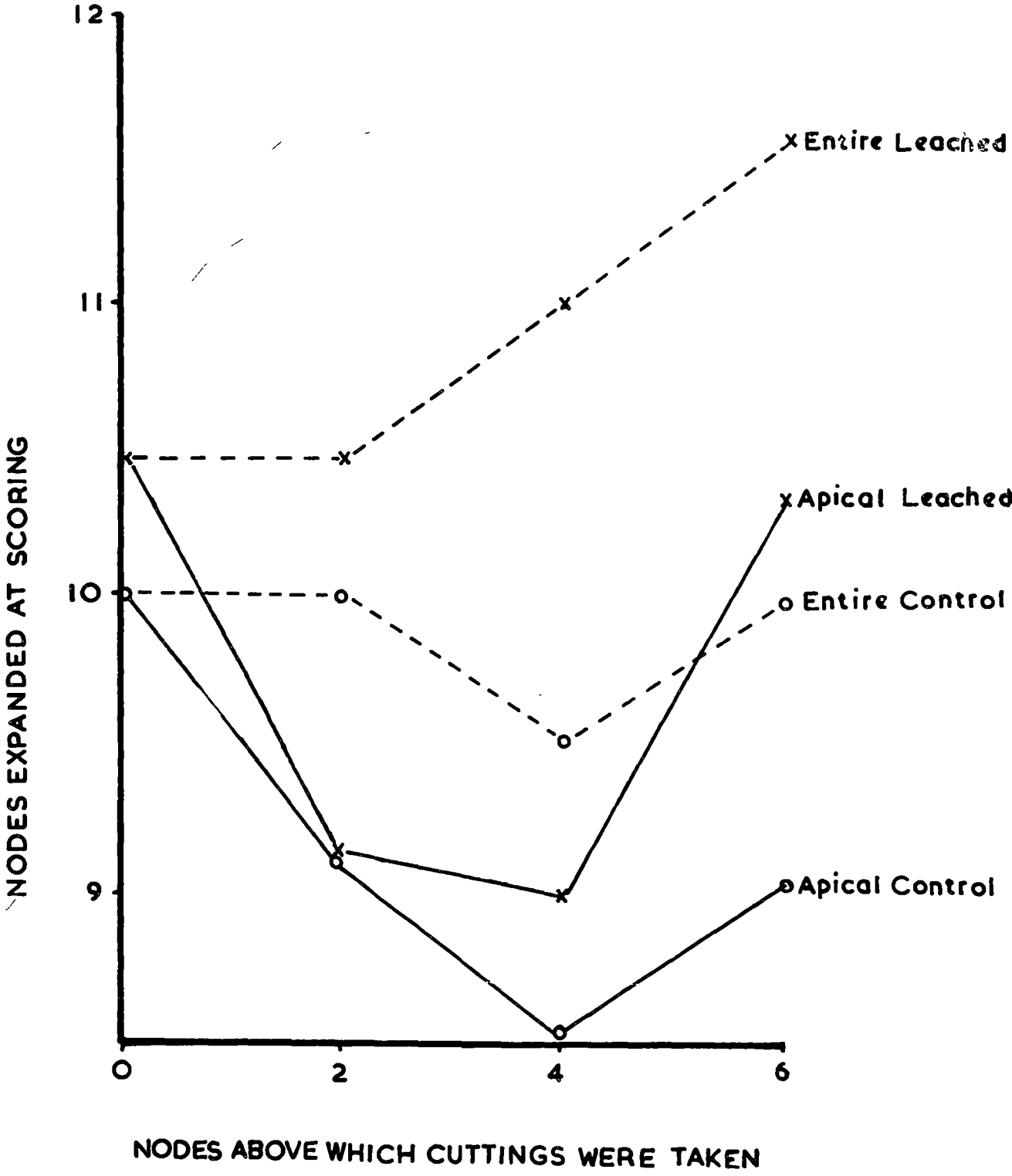


Fig. 3.3. Comparison of the effects of leaching on number of expanded nodes for entire and apical Greenfeast cuttings.

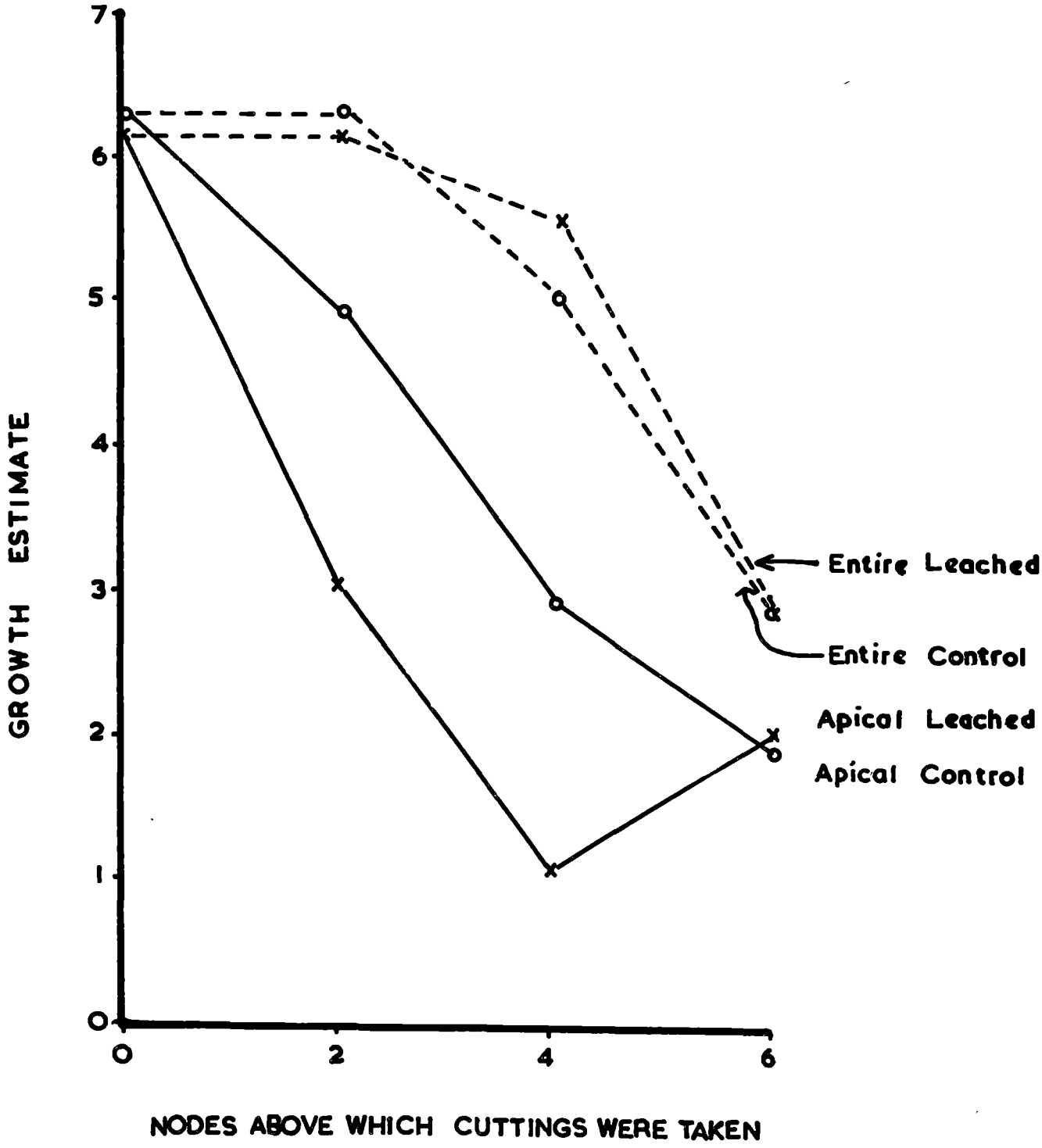


Fig. 3. 4. Comparison of the effects of leaching on general growth for entire and apical Greenfeast cuttings.

C H A P T E R 4.

EFFECT OF GIBBERELLIC ACID ON GROWTH AND DEVELOPMENT OF PEAS.

1. Introduction

Many papers have been published recently in which spectacular effects of Gibberellic Acid (GA) on various aspects of plant growth have been reported. These aspects include responses to vernalization and photoperiodism, stem growth, leaf area and morphology, and fruit production. The bulk of these papers have been summarised by Stowe and Yamaki (1957). Experiments with GA are leading to a better understanding of certain physiological processes in plants. One point that is becoming increasingly obvious is that plants of different orders, families and even genera do not necessarily show the same response to GA. Whether this implies fundamental differences between the physiological mechanisms of different plants, or just a different balance of the same components (as suggested by Brian and Hemming 1958) is not yet known.

Most reports agree that GA promotes general vegetative growth, in particular internode extension. The reported effects on leaf growth vary greatly. GA appears to promote flowering in long day plants (e.g. Arabidopsis: Langridge 1957) and those which respond to vernalization (e.g Lycopersicum: Lang 1956), when grown under non-inductive conditions. It can also replace the long day requirement in some long-short day plants (e.g. Bryophyllum: Ninnow and Harder 1956). GA can inhibit flowering even in inductive conditions in some short day plants (e.g. Kalanchoe: Harder and Ninnow 1957). Many experiments on peas have been performed by Brian and Hemming (e.g. 1958) and they have done much to elucidate the factors affecting length growth in this species. They have but few observations on other aspects of pea development. Paton (1956) performed a preliminary experiment with GA on Greenfeast peas and obtained a higher flowering node in treated plants. He suggested that this may be a direct result of an increase in the rate of node formation.

In this chapter, the variable response of pea varieties to GA will be described. Section ii gives the results of a preliminary experiment applying GA to pea seed prior to germination. Section iii, which was carried out partly in conjunction with Mr. M.D. Jackson of this department, investigates the relationships between vernalization, photoperiod and varietal response to GA. Section iv confirms and extends Brian's (1957) results with GA on the Le loci. The earlier experiments of sections ii-iv gave varying effects of GA on leaf area. In section v, GA treatments were combined with different levels of nutrient supply in attempt to find out the conditions under which GA can promote or depress growth in leaf area.

ii. Effects of Applying GA to Peas before Germination

Brian and Hemming (1955) applied GA to the first true leaf (node 3) of peas. At this stage flower initiation is taking place in early (sn) varieties. A method was therefore developed in which GA was applied to the dry seed before germination. This has been described in Chapter 1. Before this method was widely applied an experiment was performed in order to find the optimum dosage for seed treatment.

Experiment 4.1 In this experiment the peas were treated by soaking them in GA solution. Since the seeds took up different amounts of solution this method was later abandoned in favour of the one described in the introductory section. The experiment was planned as a preliminary to the photoperiod/vernalization experiment. The three varieties used were Massey, Greenfeast and Telephone. Alaska was not used as insufficient seed was available. The doses of GA received by each seed were either 0, 0.25, 0.50, 1.00, 2.00, or 4.00 µg. Seeds were planted into vermiculite in randomized blocks.

Discussion After one week's growth, when about two internodes had expanded, the plumule length of all seedlings was measured. The results are shown in table 4.1. The GA and varieties effects are significant at the 0.1% level. The interaction is not significant. It can be seen that in all varieties the maximum effect is obtained with the highest GA dose. This is in contrast to the results of Brian and Hemming (1955) who obtained maximum length increases with 1 µg applied to the leaf at node 3. It is interesting to note that Massey is the tallest variety at this stage. The differences may be chance ones as the interaction was not significant. There is no appreciable difference between the varieties when 4.0 µg of GA were applied.

Later analyses of the growth of these plants were made virtually impossible by the fact that the survival rate was very variable. This may have been exaggerated by the poor nutrient conditions. However the main source of death (usually of the main shoot only) was a result of GA application. Higher doses in particular caused a constriction in the stem just beneath the apical bud. This nearly always resulted in the death of the apex, but occasionally the plants recovered. It was therefore decided in future to restrict seed treatments to 1 µg GA where the survival rate is fairly high. The loss of plants due to GA varies with variety and treatment e.g.

Massey and Alaska vernalised plants are very susceptible. Very few plants die completely; they are usually regenerated at an early stage by means of the cotyledonary axillary buds. These results cannot be said to show that GA affects apical dominance, since the region affected is sub-apical. However the treatment results in a multiaxial plant instead of a uniaxial one, which superficially may seem to result from a loss of apical dominance. Brian and Hemming (1957a) state that GA increases apical dominance. Similar results to these found here have been described by Lang (1956b) for biennial Hyoscyamus and Langridge (1957) for Arabidopsis. These workers found that higher GA doses (10 µg/day and 3 µg respectively) resulted in some loss of apical dominance. The effective GA dosage varies widely with different species, and it seems that peas are especially sensitive to this substance.

Table 4.1

Effect of GA when applied to dry pea seed. L at 7 days.

Variety	GA/seed (µg)					
	0.00	0.25	0.50	1.00	2.00	4.00
Telephone	0.78	0.88	1.15	1.63	1.88	2.18
Greenfeast	0.75	1.15	1.00	1.73	1.95	2.35
Massey	1.33	1.33	1.58	2.23	2.30	2.45

iii. Interactions between the loci Sn and le, Photoperiod and Vernalization after GA Treatment

Brian and Hemming (1955) found that GA exerts a profound effect on dwarf (le) peas, but little or no effect on tall (Le) peas. GA has also been found to replace the light requirement of many obligate long day plants. Since peas possessing the dominant Sn gene are facultative long day plants, and Paton (1956) found that GA can cause later flowering in the variety Greenfeast, the effects of GA on all combinations of the le and Sn loci were investigated. Vernalization and photoperiodic treatments were included so as to test all known expressions of the Sn locus.

Experiment 4.2 Five factors at two levels were combined factorially. These were height (Le and le); flowering type (Sn and sn); vernalization (UV and V); daylength (SD 9 hr. and LD 16 hr.); and gibberellic acid (0 and GA). The varieties Telephone (Sn le), Greenfeast (Sn le), Alaska (sn Le) and Massey (sn le) were used for combinations of Le and Sn.

GA was applied to the dry seed in 1 µg doses as described in chapter 1, and the plants to be vernalized placed in the cold room (4 - 7°) for three weeks. A further dose of GA was given when the plants had about 4 nodes expanded, by application of solution at node 3.

Plants were arranged in 3 randomized blocks in the photoperiodic glasshouse described in chapter 1.

This experiment was exactly the same as an earlier experiment which was repeated owing to a heavy infection of eelworm. The results of the two experiments were very similar.

Plants were scored for node of first flower (F); number of expanded nodes (E); length nodes 6-2, 2-7 and 7-12 (late varieties); number of leaflets per node; leaflet area at node 6; and days to the appearance of flower buds out of their enshoething stipules.

The number of survivors in this experiment was very good (up to 48 per treatment), and complete analyses were made. Block means (for each treatment) were used as units for analysis. It was found that this gave the best estimate of the effects even when the number of plants in each unit varied slightly. The short and long day data were analysed separately. The main results are given in tables 4.2 (short day) and 4.3 (long day). The significance levels of the various treatment components are given in table 4.4. The total lengths of Greenfeast and Telephone SD plants are shown graphically in figure 4.1. Stem length was also measured over various ranges of internodes, as the varietal response to GA was found to be different over different regions of the stem.

Days to flowering was measured in an attempt to find out whether or not the effect of GA on node of first flower was a result of increased rate of node formation. It was difficult to measure as days to the appearance of abortive flowers had to be estimated.

Discussion The main effects and interactions not involving GA have been described previously for a similar experiment (Barber 1958). The results of the present experiment fully confirm Barber's data. Plants with the dominant Sn gene show a very marked flowering response both to vernalization and photoperiod. The trend for early (sn) varieties to give a small negative vernalization and long day response is also shown, but in the present case it does not reach significance. Plants with Sn respond to vernalization in both long and short days, and Barber has shown that Sn plants grown in continuous light can still show a slightly lower node of first flower when plants are vernalized. Thus it appears that not all the colysanthin is removed by long photoperiods. This has already been shown in the leaching experiments (chapter 3).

The locus Le showed no effect on flowering although the interaction Sn x Le is highly significant in both long and short days. This may well be because other loci than sn affect flowering. A polygenic and a modifying system have been suggested by Barber (1950) and the varieties used here have different components of these systems. Usually the tall varieties flower at a slightly higher node than their corresponding dwarfs. (i.e. Alaska > Massey and Telephone > Greenfeast). This effect may have been masked in the present experiment by the varietal response to GA. A glance at tables 4.2 and 4.3, shows that in both long and short days the GDV and GDU plants flower at about one node lower than the corresponding Telephone treatments. In this experiment the Massey and Alaska control plants flowered at the same node. Thus the data here confirm the hypothesis of Barber (1958) that the action of the Sn gene can be modified by other genetic systems. Recent work in this department on the F_2 generations of crosses of Le x le (Barber unpub.) suggest that the dominant Le may cause a delay of about 0.5 node in the production of first flower. This may be of interest in elucidating the flower inhibiting action of GA (see general discussion).

In general, GA treated plants have higher values of F, i.e. GA acts as some form of flower inhibitor. In the late varieties this effect is rather larger in short than in long days (figure 4.2). The effect on Massey is fairly uniform over photoperiodic

treatments, but is reduced in the V treatments. This reduction was not confirmed by Murfet (unpub.). The effect of GA on flowering in Alaska is variable. It was noticed during scoring, that this variety when treated with GA can bear flowers which abort at a very early stage. Some of these may have been missed in the early scorings. The Alaska section of the experiment was repeated and no significant effect of GA was observed. However this extra experiment was made during hot weather when the effect of GA seems to be less, so it is possible that GA may have had a real effect on P in Alaska in the earlier experiments.

In short days none of the interactions involving GA are significant. This may have been due to the larger variability in the short day plants (this is reflected in the larger values for L.S.D.). In long days many of the interactions are significant. The GA x V interaction may not reflect a real effect. In the present experiment most treatments showed a smaller response to GA when plants were vernalized. However Murfet (unpub.) failed to confirm this effect in Massey. Plants which are vernalized imbibe water at a slower rate than plants germinated in the glasshouse. This may mean that under vernalising conditions there may be time for an appreciable amount of GA to diffuse away from the seed, although it is not clear why similar diffusion did not occur in Murfet's experiment. In plants germinated in the greenhouse, very little GA diffuses away from the seed since even small doses produce a very marked response. This point requires further clarification. However, if the interaction does show a real effect and the effect of GA is greater in short days in late Sn varieties, then it suggests that GA may exert its inhibiting effect on flowering through a similar mechanism to that used by the Sn gene. Insufficient evidence has been obtained to verify this suggestion.

The GA x Sn interaction is only significant at the 5% level, and in view of the large number of significance tests made in this experiment, 5% values have been treated with caution. However if Alaska does not give a marked response to GA then it could well result in the interaction Sn x GA being truly significant.

The highly significant (0.1%) interaction GA x *la* in long days is very interesting, in view of the fact that the *la* main effect on flowering was not significant. There are two possible explanations. a) that GA has little effect on *la* plants because it is inactivated in them. This would cytochemically suggest that the GA effect on flowering would be less in tall varieties. This has been found in both long and short days. b) the effect of GA flowering may be via one of the minor systems proposed by Barber (1958). It will be shown later in the discussion that alternative a) seems the more likely.

The significant effects on days to flower vary between short and long days. This may be because of the greater variability under short day conditions. As has been mentioned, this variable was difficult to score in plants which bore abortive flowers. The main trends are similar to those observed for F, except that GA had no effect in short days. This may be because of the anomalous results of the unvernalised Telephone plants which are opposite to the general trend. No explanation for this is offered. In general, GA seems to delay the appearance of flowers by a very few days, although data is required on time to flower initiation to confirm the effect. Murfet (unpub.) has found a significant delay in time to flower initiation in Massey, but no data are available for the other varieties.

Paton (1956) from his preliminary experiment suggested that the effect of GA on F in peas may be a direct result of an increased rate of node formation. Brian (1957) found no significant effect of GA on the number of expanded nodes in the dwarf variety Meteor. An experiment carried out by final year students in this laboratory gave a small effect of GA in increasing the number of nodes formed, but this was not as great as the delay in flowering node. The varieties Massey, Telephone and Greenfeast were used. Dissections made during the course of the present experiment (when about one and three internodes were expanded) show that GA treated peas often have fewer total nodes than the control plants. This is clearly seen in table 4.5, for vernalised Massey plants. In these plants GA delayed flowering by 0.7 node but decreased the total

number of nodes by almost one. Thus in this case the delay in flowering produced by GA cannot be due to an increase in the rate of vegetative growth. Murfet has shown that this effect of GA on Massey is reversed during the later stages of growth, when the GA treated plants have a greater number of nodes than the controls. This is reflected in the greater number of expanded nodes in the GA treated plants at the time of scoring.

In the present experiment the correlation between E and F is fairly close. (It should be mentioned at this stage that E was used instead of U as the latter was biased because of longer petioles tending to cause leaves to unfold, but not expand earlier in the GA treated plants). It seems therefore, that the effect of GA on E is a secondary one, although it is very significant. Of the treatments given, GA had the greatest effect on number of expanded nodes, the effect of Sn being an artifact resulting from the early and late plants being scored at different times. The effect on vernalized plants is again rather less although the GA x V interaction is not significant.

In general the apices of GA treated plants are smaller and less well organized. This has been confirmed by sectioning methods, carried out by a student group in this laboratory. GA treated apices showed a less regular development of cells, both in L.S. and T.S.. Typical sections are shown in figures 4.9 and 4.10. The walls of the GA treated cells are more rounded, suggesting increased turgor or reduced wall strength. This lack of organization may be responsible for the early abortion of some flowers in GA treated plants. In some cases the flowers abort almost immediately after initiation. This is shown in figure 4.8. Whether or not this early abortion leads to "blind" axils is not known definitely but is suggested to account for the behaviour of some of the plants scored. The possible relationships between apical conditions and mature axils are shown in figure 4.8. The occurrence of large numbers of macroscopically abortive flowers in GA treated plants may be a result of apical disorganization, or more probably inhibition of flower development. This point will be more fully discussed later.

Massey plants have a tendency occasionally to produce two flowers in one axil. One or both of these may be abortive. GA increases this tendency in both long and short

days, particularly in vernalised plants (tables 4.2 and 4.3). Thus it seems that the effect of GA on flowering in peas is very complex. Some aspects of the system are promoted and others inhibited. However, the production of two flowers in one axil may again be the result of apical disorganisation. GA does not cause two flowers to be formed in varieties which normally never produce them.

The first node with more than two leaflets (Ca) is delayed in most of the GA treated plants. The results could be attributed to a reduction in leaf area which has the secondary effect of reducing the amount of "lobing" substance synthesised. The effect of GA in reducing leaf area was only significant at the 5% level, but this is probably due to the high variability.

Table 4.6 shows the relationship between daylength, vernalisation and GA effects on the first node with four leaflets (Cb) in late (Sn) varieties. It can be seen that both vernalisation and long days reduce this variable in control and GA treated plants. This effect is not shown by early (sn) plants. Harbor (1958) has suggested a number of pleiotropic effects of the Sn gene, one of which is control of production of leaves with more than two leaflets. If the dominant Sn gene causes plants to respond to daylength and vernalisation for both leaflet number and flowering behaviour, this would imply that the two responses (to vernalisation and photoperiod) would be governed by the same mechanism. The "lobing" substance suggested in chapter 2 could fit in with such a mechanism. The only assumption which it is necessary to make is that the "lobing" substance is available to the seedlings as soon as they have become fully imbibed, and that the passing of this substance from the cotyledons to the plumule is almost independent of temperature. An increase in temperature of from 4 to 14°C would increase the rate of physical diffusion by a factor of less than 1.4. However, the situation is almost certainly complicated by processes such as "active transport". If this assumption is valid, and since the rate of node formation in vernalised seedlings is slower than in unvernalized seedlings, the critical quantity of "lobing" substance for Ca production is reached when a smaller number of nodes is laid down. The conversion

of starch to sugar during vernalization may also help in formation of leaves with more than two leaflets. Highkin (1956) separated the effects of vernalization on flowering and vegetative growth. His results suggest two different mechanisms, but this does not exclude the possibility of both mechanisms resulting from the pleiotropic effects of the Sn gene. It is possible that "florigen" and the "lobing" factor may arise from a common precursor, of the type suggested by Sande Bakhuyzen (1947).

If "lobing" substance is produced by leaves in light, then the photoperiodic response is readily explained.

No effect of photoperiod on leaflet area was observed although few measurements were made. Paton (1956) found that leaf area in Greenfeast is larger in long days than in short days. The response of leaf area to long days varies with species, being increased in Brassica (Arney 1956) and unaffected in Trifolium pratense (Gorman 1955).

The effect of GA on shoot length has been generally found to be less in summer than in winter. The results quoted by Brian (1957) for Meteor peas grown in Britain during summer show an increase in height (with some GA dose) corresponding to the late spring/early summer figures obtained here. The temperatures in the two places at these times would be fairly similar. It may be that the higher summer temperatures in Australia lead to a smaller effect of GA. Another possibility is that GA applied to the leaves may be degraded by ultra-violet radiation, which would be stronger in summer. Yabuta et al. (1951) found that UV radiation can destroy GA in alcoholic solutions. A detailed examination of the interactions between light intensity and wave length, and temperature and GA would probably yield very useful information.

The effect of GA on stem length is different for different parts of the stem. At about 10 days after germination, tall and dwarf pea varieties are very similar in height. At this stage (nodes 0-2) GA has a marked effect on both tall and dwarf plants. The effect of GA on I_{2-7} is very marked for dwarfs and much less so for talls, and for I_{7-12} GA only has an effect on dwarf plants. It can be seen from tables 4.2 and 4.3 that the effect of GA on I_{0-2} is about twice as great in the dwarf plants as in the tall plants. This effect is general over the varieties and conditions tested. If the tall

varieties produce their natural gibberellins from a precursor present in the cotyledons, with the aid of light, then three nodes would have to be expanded for the reaction to proceed (ensuring that one true leaf is necessary to give sufficient area for photoreception). During the expansion of the first two internodes, any GA would tend to be inactivated, at least in part, if it can be utilized by the same enzyme system as the natural product. Thus tall varieties would respond to GA to a lesser degree than dwarf varieties. Over the range nodes 2-7 the tall varieties may not have reached full production of their natural GA type substance. An effect of added GA could then be obtained as here. After this, tall varieties have reached full production and added GA is either ineffective or removed by enzyme action.

If the GA is mostly removed in tall varieties, then this could account for the small effect on flower initiation in Alaska, which initiated flowers between the 2 GA doses. Sufficient may be present to cause abortion of some flowers, but not enough to delay initiation. If this GA removing system is absent from Massey (being a dwarf variety), then a delay in flower initiation could be caused by early application of GA.

Although the effect of GA on flower initiation is difficult to assess accurately because of other related effects, there seems no doubt that it can lead to flower abortion. Later experiments will amplify this point.

Table 4.2

Responses to photoperiod, vernalization, GA and their interactions in four pea varieties. Responses measured are node of first flower (F), days to the appearance of first flower (days), percentage plants with two flowers in one axil (% with 2 fls.), number of expanded nodes (E), first node with more than two leaflets (Ca) and stem length (L) between the nodes indicated.

In the treatment column, the first letter indicates variety, second letter presence or absence of GA treatment (G or 0) and third letter vernalized (V) or unvernalized (U).

SHORT DAYS (9 hours).

TREATMENT	F	Days	% with 2 fls.	E	Ca	L_{0-2}	L_{2-7}	L_{7-12}
TOU	22.03	68.27		18.87	18.43	1.10	7.63	19.33
TGU	22.83	55.53		21.57	18.80	2.77	15.90	21.40
TOV	19.67	51.47		19.70	15.73	1.37	10.03	27.53
TGV	20.73	54.27		20.23	17.27	2.67	14.57	20.43
GOU	20.97	56.80		19.93	14.07	1.03	3.40	6.47
GGU	22.43	55.30		21.53	16.83	3.67	9.90	8.80
GOV	18.77	48.17		19.67	11.97	1.20	4.03	8.60
GGV	20.67	50.20		20.43	13.90	4.33	14.90	11.40
AOU	9.67	19.80		10.73	9.63	1.07	11.60	
AGU	10.47	25.50		10.20	9.80	2.30	19.27	
AOV	9.90	20.17		10.53	9.40	1.37	10.87	
AGV	10.76	24.53		10.60	9.73	1.50	17.13	
NOU	9.87	21.57	1.0	9.43	10.17	1.50	6.13	
NGU	12.10	26.50	27.7	10.67	13.60	3.57	18.23	
NOV	9.77	22.03	2.7	9.20	10.23	1.93	6.00	
NGV	10.50	24.37	44.0	10.33	12.17	4.33	16.97	
L.S.D. 5%	0.85	5.18		1.36	0.74	0.51	2.36	
1%	1.15	6.24		1.83	1.00	0.69	3.18	

Table 4.3

Responses to photoperiod, vernalization, GA and their interactions in four poa varieties. Responses measured are node of first flower (F), days to the appearence of first flower (days), percentage plants with two flowers in one axil (% with 2 fls.), number of expanded nodes (E), first node with more than two leaflets (Ca) and stem length (L) between the nodes indicated.

In the treatment column, the first letter indicates variety, second letter presence or absence of GA treatment (G or O) and third letter vernalised (V) or unvernalised (U).

LONG DAYS (18 hours)

TREATMENT	F	Days	% with 2 fls.	E	Ca	L_{0-2}	L_{2-7}	L_{7-12}
TOU	17.30	35.53		15.50	16.13	1.03	15.43	33.73
TGU	18.37	42.30		15.37	18.13	2.63	20.23	32.70
TOV	15.00	28.90		14.70	14.27	1.67	18.50	35.10
TGV	15.40	30.67		14.97	14.47	2.73	18.40	30.23
GOU	16.10	32.07		14.40	12.23	1.00	4.53	10.73
GGU	17.17	34.63		15.33	13.50	3.30	11.43	12.97
GOV	14.40	26.07		14.40	11.13	1.17	5.03	11.47
GGV	14.87	26.00		15.67	12.23	3.60	13.47	12.93
AOU	9.67	16.27		11.47	9.57	1.03	14.43	22.50
AGU	9.83	18.37		11.60	9.80	2.13	15.30	23.03
AOV	9.83	17.40		11.57	9.30	1.53	13.30	22.90
AGV	10.37	18.80		12.07	9.63	2.67	16.10	21.03
MOU	9.57	19.49	6.7	10.50	10.30	1.23	6.83	
MGU	12.77	23.23	9.7	12.00	13.03	3.13	16.73	
MOV	9.87	18.70	2.3	10.47	10.13	2.07	7.10	
MGV	10.63	21.50	29.7	11.27	12.27	4.03	16.67	
L.S.D. 5%	0.52	2.61		0.81	0.67	0.53	3.14	5.11
1%	0.71	3.79		1.09	0.90	0.71	4.23	6.73

Table 4.4

Significance (%) of main effects and first order interactions for data given in table 4.2 and 4.3.

Variable \ Effect		GA	V	Sn	Le	GAxV	GAxSn	GAxLe	VxSn	VxLe	SnxLe
S.D.	F	0.1	0.1	0.1	n.s.	n.s.	n.s.	n.s.	0.1	n.s.	1.0
	Days	n.s.	0.1	0.1	n.s.	5.0	1.0	n.s.	0.1	n.s.	1.0
	E	0.1	n.s.	0.1	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Ca	0.1	0.1	0.1	0.1	0.1	n.s.	0.1	0.1	n.s.	0.1
	I_{0-2}	0.1	5.0	n.s.	0.1	n.s.	0.1	0.1	n.s.	1.0	0.1
	I_{2-7}	0.1	n.s.	0.1	0.1	n.s.	5.0	0.1	1.0	n.s.	n.s.
	I_{7-12}	n.s.	0.1	-	0.1	0.1	-	1.0	-	n.s.	n.s.
I.D.	F	0.1	0.1	0.1	n.s.	0.1	5.0	0.1	0.1	n.s.	0.1
	Days	0.1	0.1	0.1	n.s.	1.0	n.s.	n.s.	0.1	n.s.	0.1
	E	0.1	n.s.	0.1	1.0	n.s.	n.s.	1.0	n.s.	n.s.	n.s.
	Ca	0.1	0.1	0.1	0.1	n.s.	n.s.	0.1	0.1	1.0	0.1
	I_{0-2}	0.1	0.1	n.s.	0.1	n.s.	n.s.	0.1	5.0	n.s.	1.0
	I_{2-7}	0.1	n.s.	n.s.	0.1	n.s.	n.s.	0.1	n.s.	n.s.	0.1
	I_{7-12}	n.s.	n.s.	-	-	n.s.	-	-	-	-	-

Table 4.5

Effect of Ga on node formation in vernalized Massey peas. Means of 7 readings with negligible variability.

	Number of Exp.nodes	Nodes unfolded	Nodes Clasping	No Primordia	Total nodes	F
GA	4.3	0.7	4.1	2.9	12.0	10.4
Control	4.4	0.4	4.3	3.7	12.8	9.7

Table 4.6

Effect of GA, vernalization and daylength on node of first four leaflet leaf in late (Sp) varieties.

Treatment	LD	SD
GCU	12.23	14.07
GCV	11.13	11.97
GGU	13.50	16.83
GGV	12.23	13.90
TCU	16.13	18.43
TCV	14.27	15.73
TGU	18.13	18.80
TGV	14.47	17.27
L.S.D. 5%	0.67	0.79
L.S.D. 1%	1.90	1.00

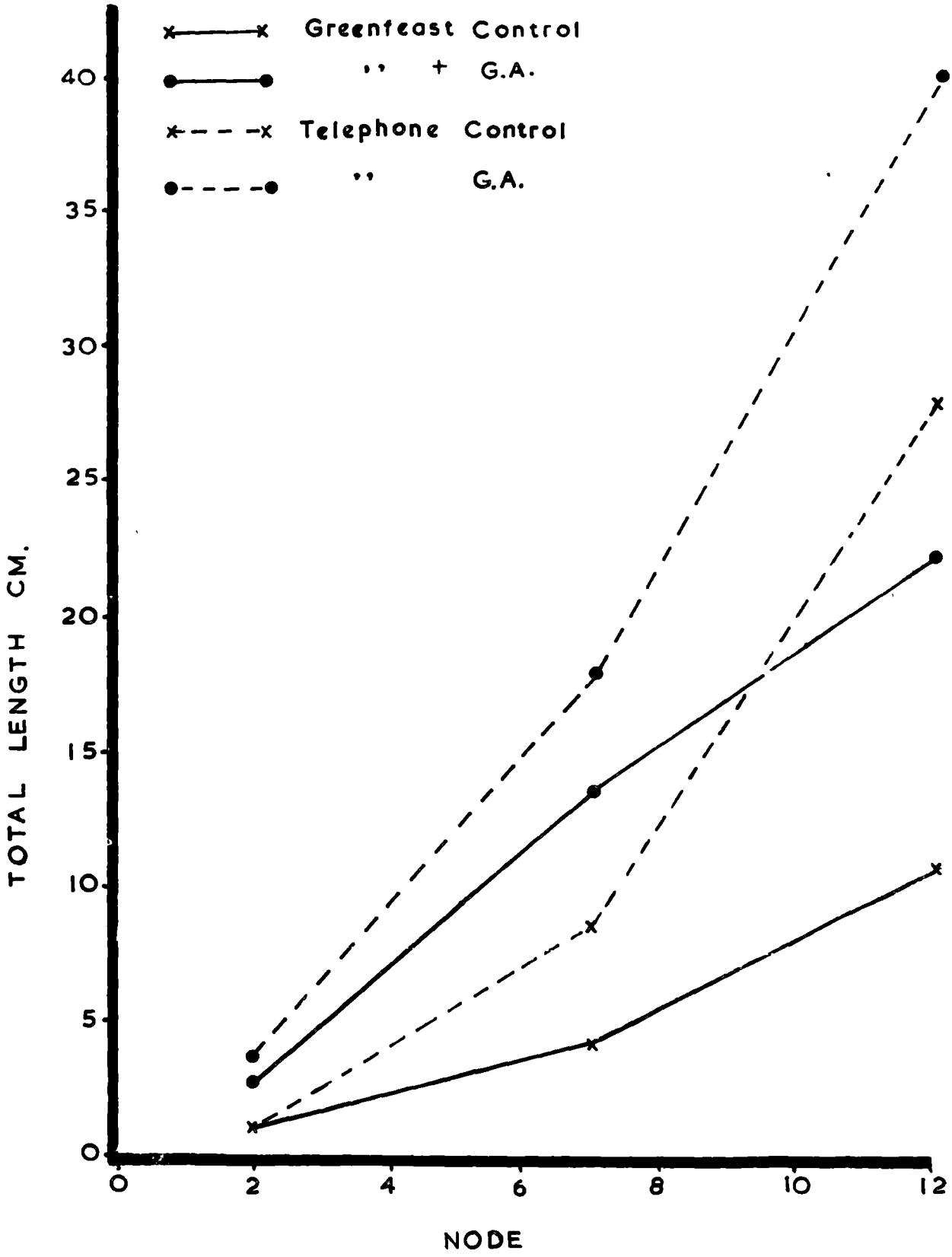


Fig. 4.1. Effect of GA on total length of a tall (Telephone) and a dwarf (Greenfeast) variety. Data from short day, unvernalized plants, is given in table 4.4.

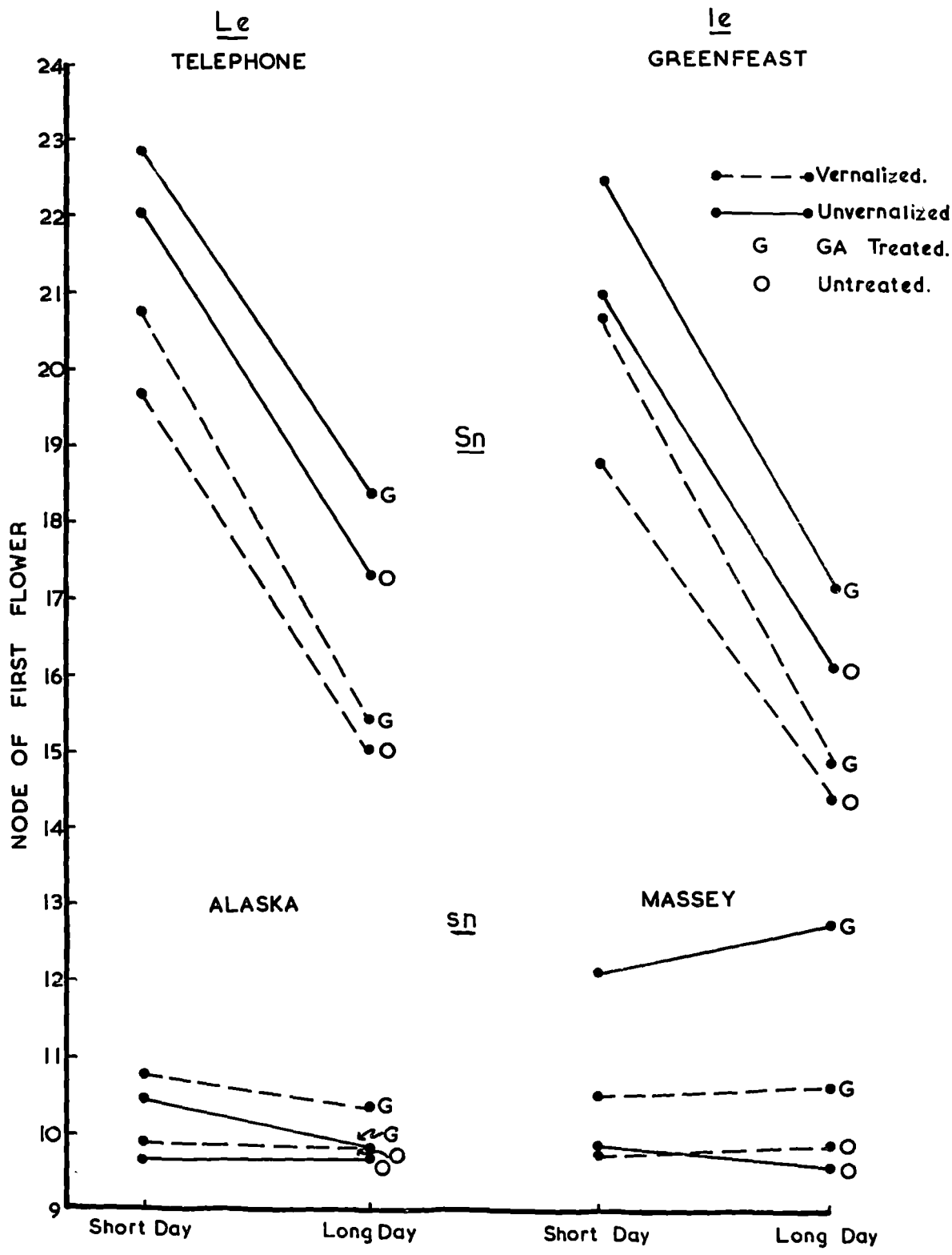


Fig. 4.2. Effects of short (9 hours) and long (18 hours) days on flowering in Telephone, Greenfeast, Alaska and Massey peas given various vernalization and photoperiodic treatments.

iv. Interactions between GA and the Cy loci.

A brief description of the action of the Cy loci on pea internode length has been given in chapter 1. In view of the marked effect of GA on dwarf (le) peas (Brian and Hemming 1955), it seemed interesting to test the effects of GA on the Cy loci. Since the first of these experiments was performed, Brian (1957) has tested some of the known Cy genotypes with GA. He found no effect on the slender (cy_1 cy_2^s) type, and suggested that the effect of GA on the other genotypes was to remove the inhibition of internode elongation caused by the dominants Cy_1 and Cy_2 (Lamm 1937). This hypothesis has been fully confirmed by the work described in this section. Unfortunately, all combinations of the two Cy loci were not available, so that the experiments had to be restricted. In the first experiment indole acetic acid (IAA) was also applied for comparison, and in the second, maleic hydrazide and 2, 6-diaminopurine were used. The former substance was found by Paton (1956) to reduce the flowering node in peas and the latter has been shown to inhibit cell enlargement (Miller 1953, Satterfield and Duncan 1955).

Experiment 4.3 In this experiment the following lines were used.

De Ruan	3 lole	cy_1 cy_1 Cy_2 Cy_2
De Ruan	6 lole	Cy_1 Cy_1 cy_2^s cy_2^s
"acacia"	7 lole	Cy_1 Cy_1 cy_2^s cy_2^s
cryptodwarf	8a lole	cy_1 cy_1 cy_2^c cy_2^c

It can be seen that lines 6 and 7 have the same Cy complement. However they differ in many other respects e.g. line 7 (tl tl) has all tendrils replaced by leaflets. It also has a very low node of first initiated flower (about 6) and has small white seed (as opposed to the large brown mottled seed of line 6). It also produces a number of abortive flowers, the node of first functional flower depending on environment. Any differences in response between lines 6 and 7 may show how GA interacts with other factors inside the plant. Lines 3 and 6 are essentially the same, except for their Cy complement. They both produce strong plants and have a slight tendency to go "blind" in the apex. Line 8a has white, wrinkled seed and shows the typical semi-tall habit of the "cryptodwarf" peas. When this experiment was commenced, no information was available on the flowering nodes of lines 3, 6 and 8a. GA was therefore applied

to node three as in Brian and Hemming's early work. This was afterwards found to be too late since all lines behaved as early peas. Indole acetic acid (IAA) was applied in a similar fashion. The following treatments were made on each of the four lines

none (control)
2 µg IAA
2 µg GA
2 µg IAA + 2 µg GA

The dosages were chosen after reference to the papers of Von Abrams (1953) and Brian and Hemming (1955). The number of replicates was limited by the number of seed available. Four randomized blocks, each with units of 4 plants were used, except for line 8a when the units were of 3 plants. Plants were given natural daylight supplemented to give an 18 hour day. They were scored for F, Ca and Cb. The internode length was measured between nodes 3 and 8 as all plants had 8 nodes expanded and this region was thought to give a good measure of the effects of the substances applied. Leaflets and stipules from node 6 were measured for area. This node was chosen as the GA effect was maximal at this stage and the number of leaflets was relatively stable between lines 3, 6 and 8a and within line 7. Other workers had found no effect of GA on node expansion and the effect in this experiment was not realized until scoring was well under way. Figures are only available for 2 replicates, but they give significant effects. The number of unfolded leaves was measured, probably giving a slight over estimate of the effect of GA since it was later realised that GA causes petioles to extend slightly, thus causing leaves to unfold, but not expand, earlier.

The results are summarized in table 4.7. Since all combinations of the Cy genes were not available, their effects could not be subdivided statistically. The data for F were not analysed since there was obviously no effect of GA. However, GA had a marked effect on the node of first functional flower. The effect of GA on internode length (figure 4.3) was very large, but differed significantly between varieties. Leaflet area at node 6 was reduced by GA, but stipule area was unaffected. The number of unfolded leaves showed a significant increase in GA treatments. In no case did IAA have a significant effect when the results were analysed as a whole.

Experiment 4.4 In this experiment lines 3, 6 and 7 were used, to confirm the effects of GA on these lines and in particular the difference between its effects on lines 6 and 7. Insufficient seed of line 8a was available. 2, 6, diaminopurine (DAP) and maleic hydrazide (MH) were included for comparison on line 3. Maleic hydrazide has the superficial effect of reversing internode extension and other effects of GA (Bukovac and Wittwer 1956) although Brian and Hemming (1957) concluded that the effects of the two compounds were on different metabolic steps. 2, 6-diaminopurine has been showed to affect leaf expansion (Liverman and Scott 1957).

Plants were treated before germination with 2 µg of GA in alcoholic solution, or one drop (about 2 µg) of a suspension of MH or DAP. One series was treated with MH + GA. A second, similar, treatment was applied when the plants were about 10 days old. Plants were arranged in a similar design as in experiment 4.4, but with units of 5. They were grown under natural long days (about 16 hrs.).

The plants were scored early so that flower abortion could not be measured. Owing to the earlier application of the chemicals, leaflets and stipules from node 4 were measured for area, and internode length was measured between the cotyledons (node 0) and node 5. Node of first initiated flower, number of

expanded nodes and leaf thickness (node 4) were also measured. The early scoring prevented any measurements of Caboing made. Leaf thickness was measured with a micrometer gauge.

The results are summarized in table 4.10. No significant (1% level) treatment effects on F were observed. The flowering behaviour of line 3 was unaccountably variable, but the difference only just reached the 5% significance level. The effects of GA and MH appear to be the reverse of expected, and found by Paton (1956) using Sn plants. GA caused a significant increase in E, but MH and DAP had no effect. Stipule area was not significantly affected by treatment but GA significantly reduced leaflet area. These two variables had large variances. GA resulted in the usual increase in internode length. All chemical substances caused a decrease in leaf thickness, but the effect of GA was the greatest.

Discussion In neither experiment did GA have a significant effect in delaying flower initiation. In experiment 4.3 GA was only applied at the time of flower initiation, but in experiment 4.4 application was made and was fully effective well before initiation in lines 3 and 6.

The effect of GA on internode length was highly significant in both experiments. IAA had no effect on any line with the possible exception of 3. In this case IAA caused an increase in all replicates and it was significant at the 5% level when this line was analysed separately (see table 4.9 for analysis). Whether or not this represents a true effect of IAA is not certain, but the increase is of a similar order to that found by von Abrams (1953) and in some of the experiments of Brian and Hemming (e.g. 1955). In view of the latter authors' most recent paper (Brian and Hemming 1958) IAA is not normally likely to be limiting, and this probably accounts for lack of effect of IAA in this experiment. None of the substances other than GA used in experiment 4.4 had an effect which was significant at the 1% level.

The length response of the different lines to GA varies widely. The nett effect of GA on the different lines (i.e. GA values - control values) in experiment 4.4 is given in table 4.8. It can be seen that the GA effects on lines 3 and 6 are very similar. This is true also for experiment 4.4. In both experiments, the response of line 7 is less than that for line 6. These two lines have the same complements of the length loci $\underline{le}$, $\underline{Cv_1}$ and $\underline{Cv_2}$. It appears therefore, that some other factor is limiting. The responses of the two lines can be much more nearly equated if expressed

as a proportion of the normal length (i.e. GA value divided by control value). In this way, the response of line 7 is larger than that of line 6 in experiment 4.4 and line 6 has the larger response in experiment 4.3. The most likely cause of discrepancy between the absolute responses of the two varieties is nutrient supply. Line 6 has much larger seed and this may provide a greater amount of food to the young plant than line 7. This shows that care must be taken when comparing GA effect on different varieties. Table 4.8 and figure 4.3 show that the length response of line 8a to GA is much less than that of the other lines. This is to be expected since the line has two recessive Cy genes. The "slender" line (cy, cy_2^s) has been shown by Brian not to respond to GA. The normal expression of cy_2^c is intermediate between Cy_2 and cy_2^s. This is confirmed by its intermediate response to GA. The present results also confirm the hypothesis of Brian and his co-workers on the mode of action of GA on length growth in peas. Lamm (1937) showed that the inhibitory action of the two Cy genes is not equally strong, Cy_1 having a stronger effect than Cy_2. The control plants of lines 3 and 6 which bear these dominant genes were exactly the same height. However, the relative increase in height of GA treated plants compared with control plants was greater in line 6 which bears the dominant Cy_1. This is to be expected if Cy_1 has a stronger action than Cy_2. The lack of difference between the control plants of the two lines may have been due to later scoring: Lamm measured his plants when only 5 nodes were visible, whereas twice this number was visible in the plants of the present experiment.

After this experiment was completed, a further length mutant ("micro" lm lm) was discovered (Lindqvist 1951). It would be of interest to test this locus with GA, particularly in view of the report of Phinney (1956) that nanomize mutants do not respond to GA.

In both experiments GA resulted in a significant increase in E (or U). The increases in the two experiments were of the same order, about one node extra for every 12 nodes expanded on the control plants. A detailed explanation of these results cannot be given, as no dissections were made during the growth of the plants, to see whether the

the effect of GA took place after flowering or during the whole period after treatment.

In experiment 4.3, the interaction lines X IAA is significant at the 5% level (for U). An inspection of the treatment means shows that IAA has no effect on lines 6 and 7, increases U in line 3 and decreases it in line 8a. The last two opposite effects could mask the significance of the main effect and yet show up in the interaction. This point has been discussed by Cochran and Cox (1950 pp. 139-141). They suggest the use of "t" tests in the appropriate cases. In this data L.S.D. values are given, which are equivalent to a "t" test. Use of L.S.D. values show no significant differences in the present data and therefore the lines X IAA interaction has been ignored. However it is worth bearing in mind that the effects of GA and IAA on lines 3, 6 and 8a are additive, as can be seen by comparison of individual effects with the GA + IAA treatments.

There was no significant effect of treatment on Ca in experiment 4.3. In lines 3 and 6 the first node with more than 2 leaflets was determined when GA was applied. There was a slight delay in GA treated plants of line 8a, but this was not significant. In this line Ca was determined very soon after GA application.

There was a marked effect of GA in reducing leaflet area in both experiments. In experiment 4.3, the lines x GA interaction is significant. An inspection of the data in tables 4.7 and 4.10 shows that the reduction in leaflet area is largely confined to lines 3 and 6. These plants naturally have larger leaflets than those of lines 7 and 8a and they seem to be more readily affected. The effect of GA on leaf area is obviously a complex one, as Stowe and Yamaki (1957) have shown, different varieties reacting very differently.

There was no effect of GA on stipule area. This may be because the variance was much greater than for leaflet area. It is also possible that the vascular supply to the stipules is less efficient than that to the leaflets and thus less material reaches them to have an effect.

Table 4.10 shows that GA has a highly significant effect in reducing leaflet thickness. This effect was not shown so markedly by any other chemical substances tested (i.e. DAP and HH). Unlike the effect on leaflet area, GA also reduces leaflet thickness in line 7. It appears that under normal glasshouse conditions, GA reduces all

aspects of leaf growth. Whether this is connected with auxin metabolism or not is unknown. It is not impossible since auxin-type substances are known to cause reductions in leaf area, although these are usually accompanied by distortion and stimulation of midrib development.

Table 4.7

Effect of GA and IAA on the *Cy* loci.

Data for nodes of first initinted flower (F) and first functional flower (Ff) first node with more than two leaflets (Ca), length nodes 3-8 (L_{3-8}), area of stipules (AS6) and leaflets (AL6) at node 6, and the number of unfolded nodes (U). The lower half of the table shows the significance levels for the various treatments (n.s. = not significant).

Treatment or effect	F	Ff	Cb	L_{3-8}	AS6	AL6	U
line 3 control	11.20	11.95	10.08	13.25	8.38	14.77	9.5
GA	11.90	13.23	9.65	32.48	8.43	12.24	10.7
IAA	12.20	12.33	9.83	14.73	8.81	13.54	10.2
GA + IAA	11.95	13.25	9.78	35.40	8.15	11.99	11.0
line 6 control	10.33	11.60	9.90	13.25	9.29	14.58	9.9
GA	10.25	12.33	9.68	36.45	9.27	11.69	10.4
IAA	10.53	11.28	10.08	13.63	9.11	14.25	10.2
GA + IAA	10.95	12.25	10.00	36.58	8.46	11.52	11.2
line 7 control	6.28	8.63	-	11.70	5.52	8.34	8.2
GA	6.40	11.75	-	27.95	5.41	8.33	10.3
IAA	6.53	10.13	-	11.18	5.07	8.48	8.2
GA + IAA	6.30	10.88	-	25.25	5.24	8.36	9.9
line 8a control	11.38	12.43	11.93	19.63	3.48	5.03	11.2
GA	11.25	12.38	12.38	30.40	3.28	4.59	12.0
IAA	11.03	11.50	11.53	20.10	3.19	4.63	10.7
GA + IAA	11.13	12.38	11.88	30.13	3.22	4.33	11.3
lines		0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
GA		0.1%	n.s.	0.1%	n.s.	0.1%	0.1%
IAA		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
lines x GA		1.0%	n.s.	0.1%	n.s.	5.0%	5.0%
lines x IAA		n.s.	n.s.	10.0%	n.s.	n.s.	5.0%
GA x IAA		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
lines x GA x IAA		0.1%	n.s.	n.s.	n.s.	n.s.	n.s.
L.S.D. 5%		0.77	1.00	2.80	1.12	1.99	0.73
L.S.D. 1%		1.03	1.36	3.76	1.48	2.67	1.01

Table 4.8

Effect of GA on varieties differing in the Cy loci.

(cases where the interaction is significant)

Data calculated from table 4.7.

Line	Variable			
	Ff	L_{3-8}	AI_6	U
3	+1.3 nodes	+19cm.	$-2.5cm^2$	+1.2 nodes
6	+0.7 nodes	+23cm.	$-2.9cm^2$	+0.5 nodes
7	+3.1 nodes	+16cm.	$\pm 0.0cm^2$	+2.1 nodes
8n	-0.1 nodes	+11cm.	$-0.5cm^2$	+0.8 nodes

Table 4.9

Analysis of Variance for L_{3-8} in line 3 alone.

Effect	ss	df	ms	F ratio	sig.level
Blocks	46.35	3	15.45	4.53	5.0%
GA	1592.01	1	1592.01	466.87	0.1%
IAA	19.36	1	19.36	5.68	5.0%
GA x IAA	2.11	1	2.11	0.62	n.s.
Error	30.65	9	3.41		

Table 4.10

Effects of GA, Maleic hydrazide (MH) and 2,6, diamino-purine (DAP) on growth of 3 pea genotypes

Data for node of first flower (F), number of expanded nodes (E) area of stipules (AS_4) and leaflets (AL_4) at node 4, length from cotyledons to node 5 (l_{0-5}) and leaf thickness at node 4 (Th_4).

Line	Treatment	F	E	AS_4	AL_4	l_{0-5}	Th_4
3	0	10.07	5.80	4.27	8.49	5.8	0.320
	GA	9.48	6.10	4.21	6.79	19.9	0.239
	DAP	9.85	5.50	3.95	8.43	7.0	0.287
	MH	10.12	5.85	4.96	8.97	9.5	0.282
	GA + MH	9.60	6.05	3.53	5.55	19.0	0.241
6	0	9.75	5.30	5.09	10.53	6.5	0.330
	GA	9.98	6.10	4.66	7.63	18.0	0.234
7	0	6.45	5.82	2.59	5.22	3.6	0.320
	GA	6.40	6.48	3.00	4.25	11.9	0.241
L.S.D.	5%	0.58	0.25	3.01	2.27	3.23	0.031
	1%	0.79	0.34	4.08	3.08	4.38	0.042

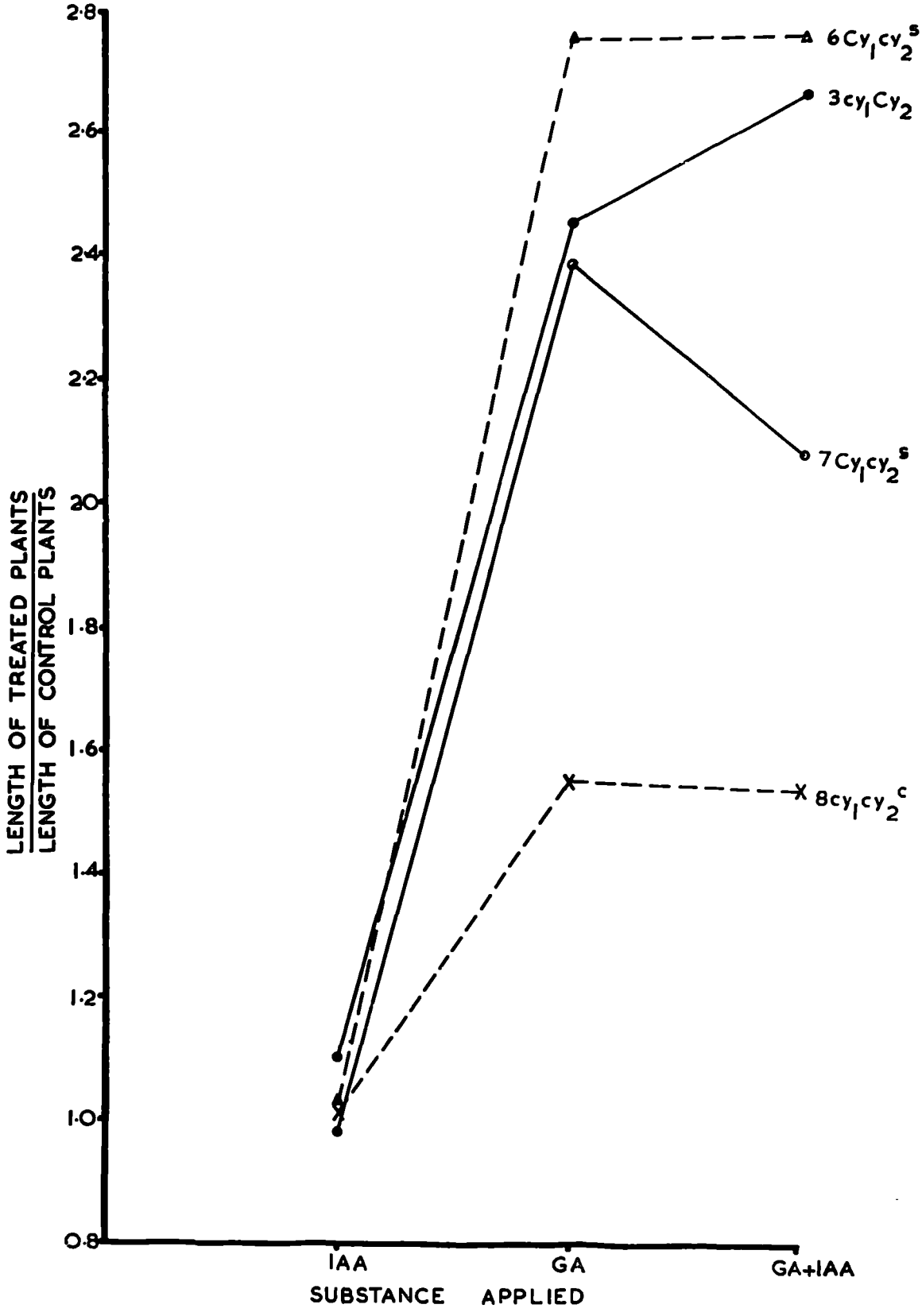


Fig. 4.3. Effect of GA and 1AA on the Cy Loci

v. Interaction between dose and time of GA application and nutritional environment of plants.

During the course of the present studies, it has been noticed that the response of plants to GA varies. In particular the effect of GA on leaf growth seems variable. In most experiments GA application results in reduced leaf area, but in one (section vi) there was a slight increase. This variability in response has also been discussed by Stowe and Yamaki (1957). It seemed that the most likely cause of reduction in leaf area was limiting nutrient supply. This could occur in both the internal and external environment of the plant. To test the hypothesis that internal nutrient supplies are limiting, GA was applied in different concentrations, and at different nodes. Thus the amount of food reserves and photosynthetic area per unit of GA was varied in two ways. The external environment was one of two types, standard potting compost (organic) and a vermiculite/gravel mixture (inorganic) given only a minimal amount of nutrient solution.

Experiment 4.5. The general plan of the experiment has been given above. The following treatments were combined factorially

4 dosages, 0, 1, 10 and 100 µg GA in alcoholic solution
3 nodes of application, nodes 3, 6 and 9
2 nutrient supplies, organic and inorganic (1 part vermiculite: 4 parts dolorite chips)

Plants were grown in units of 5 per tin, 24 tins being grouped in one randomised block. 4 blocks were used, and plants were grown under natural long days (about 16 hours). The experimental design enabled the nutrient treatments to be fully randomised within blocks.

The following variables were scored.

F
E

Days to appearance of flower buds out of ensheathing stipules

Ga
I_{3-6}
I_{6-9}
I_{9-12}

Leaflet area at nodes 7 and 10
Thickness of leaflets at node 10
Occurrence and number of abortive flowers
Number of dead leaves (including scale leaves).

The figures for leaflet area at node 7 were variable, probably because some leaves were not fully expanded. They were not analysed. All other measurements taken were analysed, missing plots being estimated according to the procedure set out by Gauldon (1952). Table 4.11 gives the figures for all variables except length, area and occurrence of abortive flowers. The latter are shown graphically in figures 4.4, 4.5 and 4.6. The effects of GA on flowering can be seen to be fairly small and some of the length effects are not as great as previously found. Otherwise the figures confirm and extend those already given in this chapter. Leaf thickness data for node 6 applications are shown in figure 4.7.

Discussion A delay of up to one node in F was obtained by the application of GA, the larger doses giving the greatest effect. The effect of 1 µg was smaller than usual and the only explanation offered for this at the moment is that the weather conditions (e.g. temperature, ultra-violet radiation) affected the response to GA. Application of GA at node 3 had the effects noticed before, while application at node 9 had no effect on F. This is because all plants had initiated flowers at the time of application. The response to GA applied at node 6 varies with the nutrient treatment. In organic nutrient, GA causes a delay in F, but in inorganic it has no effect. This is because the plants in the latter nutrient treatment have initiated flowers. In organic nutrient, the plants are at the point of "ripeness to flower", but GA still causes a delay in initiation. This cannot be explained by an increase in the rate of node formation. The largest effect of treatment on F in this experiment was due to the different nutrient conditions. The inorganic nutrient plants flowered at node 12.97 whereas the soil grown plants flowered at node 14.35. The inorganic nutrient plants also flower about three days earlier than the soil grown plants, which suggests that the difference in F is not a result of vegetative growth differences. In three days about 1.5 nodes are laid down (Paton and Sprent unpub.) which is equal to the difference in F. This difference must therefore be due to the presence in the inorganic series of a system tending to promote flowering, or alternatively, an inhibitory system in the organic serie

The exact nature of such a system is not known. It may be relatively simple. For example, mineral nutrients (e.g. K and P) may alter the rate of senescence of leaves (Watson 1947 and 1956). The leaves of the inorganic series have been found to die more rapidly than those in soil (table 4.12). This may reflect a faster metabolic rate and consequently a faster removal of flower inhibiting substances in the leaves.

A detailed examination of the effect of treatment on days to flower was not possible because of the high variability and the difficulty of scoring abortive flowers. The only reliable result is the accelerating effect due to plants being grown in inorganic nutrient.

The percentage of plants with abortive flowers (figure 4.6) shows that GA can inhibit the process of flower development. Only the data for the inorganic series are shown in the figure, because they were more complete, but the organic figures are similar. In all cases where GA was applied after flower initiation one or more abortive flowers were formed. This is true both when GA is applied close to the time of initiation (i.e. at node 6) or several days after initiation (node 9). In the latter case, plants produce one or more normal flowers followed by one or more abortive ones. In the extreme treatments (100 µg) the percentage of plants bearing abortive flowers reaches 100. No abortion occurred in normal untreated plants, or in those treatments where GA was applied well before initiation (node 3). These results can be compared with those of Harrington et al. (1957) who found that GA induced the initiation of flowers in endive, but these flowers were abortive. This is a long day plant with a vernalisation requirement. In vernalized plants the authors found that GA delayed bud formation until much elongation had occurred. Most of the flowers formed were abortive until the GA supply ran out.

The present experiment indicates that there is a particular time in the development of the flower when it is especially sensitive to GA. When GA is applied after the commencement of flower initiation only flowers at this critical stage of development are inhibited. This is shown in the plants which had GA applied at nodes 6 and 9.

Applications at node 6 were made just as flower initiation was beginning and the first few flowers formed were abortive. When GA was applied at node 9, the first few flowers developed normally, but those which were being initiated at the time of treatment were inhibited. Thus it appears that GA cannot effect the development of flowers after they have reached a certain stage. The only flowers to be affected are those which are being initiated at the time of GA application. This adds weight to the suggestion made earlier that the effect of GA on flowering may be only a secondary result of disruption of the apical organization.

The results presented in this thesis indicate that the effects of GA on flowering may be divided into two distinct types. These are (a) delaying (or promoting in some plants) of flower initiation and (b) inhibition of flower development. In peas, both effect may be a result of apical disorganization. This point will be more fully discussed in chapter 5.

The number of expanded nodes shows a steady increase with increasing GA dose, the effects being the same in both nutrient treatments. The means for the dosage treatments are 12.44, 12.79, 13.58, and 13.98 for 0, 1, 10, and 100 µg respectively. The effect of node of application is also significant, the means being 13.50, 13.11 and 12.98 for applications at nodes 3, 6 and 9. None of the interactions reached the 1% level of significance, so that the means represent a fairly homogeneous treatment effect. The number of expanded nodes is not highly correlated with the node of first flower, except that the effect of increasing GA dose is the same on these two processes. There was no effect of nutrient on E although this treatment had a highly significant effect on F. The effect of node of application on E is probably a direct result of the different times between GA treatment and scoring. In agreement with the results of other experiments, GA in this case had a quantitative effect in increasing the number of expanded nodes.

The node of first leaf with more than two leaflets shows the usual, though smaller, delay in GA treated plants (node 3 series). The leaf pattern had been

determined when the applications were made at nodes 6 and 9 and this accounts for the lack of GA effect on these plants. Plants grown in inorganic nutrient have a lower value (11.01) than the organic series (11.40), and this effect is significant at the 0.1% level. This again is suggestive of a faster metabolic rate, in the plants grown in inorganic nutrient. It may also be a result of slightly larger leaf area (7.74cm^2) in inorganic compared with organic (7.17cm^2). This effect was not significant. The figures for Ca in this experiment cannot be directly correlated with variations on the rate of node formation. None of the treatments had a marked effect on the reversion from leaves with a larger, to leaves with a smaller number of leaflets.

The results for leaflet area at node 10 are very interesting although not entirely as expected. All the main effects and first order interactions are significant, with the exception of "nutrient". All the data except that for applications at node 9 (which had no effect) are shown graphically in figure 4.5. It can be seen that the range of values is much greater in inorganic than in organic nutrient. All the curves seem to be reaching a uniform minimum area for this node at 4-5cm^2. It can be seen that the effect of GA on area is much greater in inorganic than in organic nutrient. In inorganic nutrient the effect of GA applied at node 3 is more marked than at node 6. The reverse is true for organic nutrient although the effect of GA here is much smaller. Under the conditions of this experiment the medium in which the plants were grown was not the limiting factor for leaf area development, although this had been the intention. Leaf area in the plants grown in inorganic nutrient was much more readily affected by GA applications than leaf area in soil grown plants. Although none of the GA treatments resulted in an increase in leaf area, it is quite clear that the effect of GA varies with environment. GA causes a greater reduction in leaf area in plants grown in inorganic nutrient than in those grown in soil. This is to be expected, since Brian et al. (1954) found that the effects of GA are intimately related to the amount of fertilizer applied.

The number of dead leaves was only affected by two of the treatments applied. It was greater (6.59) in inorganic nutrient than in organic (6.12). GA doses of 10 µg and over also increased the number of dead leaves by up to 1.5 nodes. This is of the same order as the increase in E with GA and thus it seems that the total number of functional leaves which can be supported by the plant is fairly constant (i.e. E minus number of dead leaves is a constant).

In this experiment some of the GA treated plants (particularly in inorganic nutrient)had flatter leaflets with entire margins. This is in agreement with the observation of Gray (1957) on tomtoes.

The length data are incorporated in figure 4.4. They are largely the same as those found by Brian and Hemming (1955), the larger doses giving a more prolonged effect than small doses. The response to GA is generally greater in inorganic than in organic grown plants. The data also show that GA has no effect on mature internodes. It can be seen that very few of the plants receiving 1 µg gave a response. This result was very surprising, since this quantity usually gives the maximum response over a limited number of internodes.

Table 4.11

Effect of nutrient, GA (0, 1, 10 or 100 µg, as indicated by first figure) applied at different times (nodes 3, 6 or 9 as indicated by second figure) on growth of Greenfeast pea. Data for node of first flower (F), days to flower (days), first node with more than 2 leaflets (Ca), number of expanded nodes (E) number of dead leaves and leaf thickness at node 10 (Th10).

Treatment	F	Days	Ca	E	Dead leaves	Th10
GM. 0 3	14.43	9.08	11.23	12.93	5.75	0.220
1 3	14.53	8.68	11.35	13.15	5.63	0.240
10 3	14.48	6.35	11.88	14.13	6.45	0.254
100 3	15.58	9.00	12.23	14.65	7.08	0.240
0 6	13.95	9.98	11.10	12.00	5.63	0.219
1 6	14.33	10.25	11.43	12.63	5.70	0.223
10 6	14.18	8.05	11.38	13.53	6.25	0.186
100 6	15.05	11.20	11.40	14.00	6.50	0.180
0 9	13.75	8.75	11.15	12.20	5.90	0.226
1 9	14.75	11.18	11.75	12.68	5.90	0.204
10 9	13.78	7.25	11.00	13.33	5.95	0.213
100 9	13.40	6.03	10.90	13.43	6.75	0.196
INGM. 0 3	12.65	5.93	10.53	12.60	6.50	0.219
1 3	13.33	6.38	11.25	12.80	5.68	0.229
10 3	13.15	5.58	11.43	13.50	6.53	0.198
100 3	13.55	5.95	11.43	14.25	8.05	0.184
0 6	12.95	6.35	10.73	12.38	5.90	0.205
1 6	12.90	5.98	10.80	12.83	6.15	0.205
10 6	12.93	6.08	11.15	13.53	6.35	0.158
100 6	13.08	4.75	11.18	13.98	7.20	0.152
0 9	12.75	6.10	10.85	12.55	6.30	0.225
1 9	12.45	5.45	10.65	12.65	6.60	0.201
10 9	12.85	4.68	11.00	13.48	7.00	0.219
100 9	13.03	5.05	11.20	13.55	6.88	0.213
		SIGNIFICANCE LEVELS				
DOSE GA	1.0%	5.0%	0.1%	0.1%	0.1%	1.0%
GA at NODE	0.1%	n.s.	0.1%	0.1%	n.s.	0.1%
NUTRIENT	0.1%	0.1%	0.1%	n.s.	0.1%	0.1%
D X NO.	n.s.	n.s.	5.0%	n.s.	n.s.	1.0%
D X NU	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
NO X NU	n.s.	n.s.	n.s.	5.0%	n.s.	1.0%
D X NO X NU	1.0%	n.s.	5.0%	n.s.	n.s.	n.s.
L.S.D. 5%	0.71	2.67	0.16	0.68	0.80	0.029
L.S.D. 1%	0.84	3.19	0.19	0.81	0.96	0.035

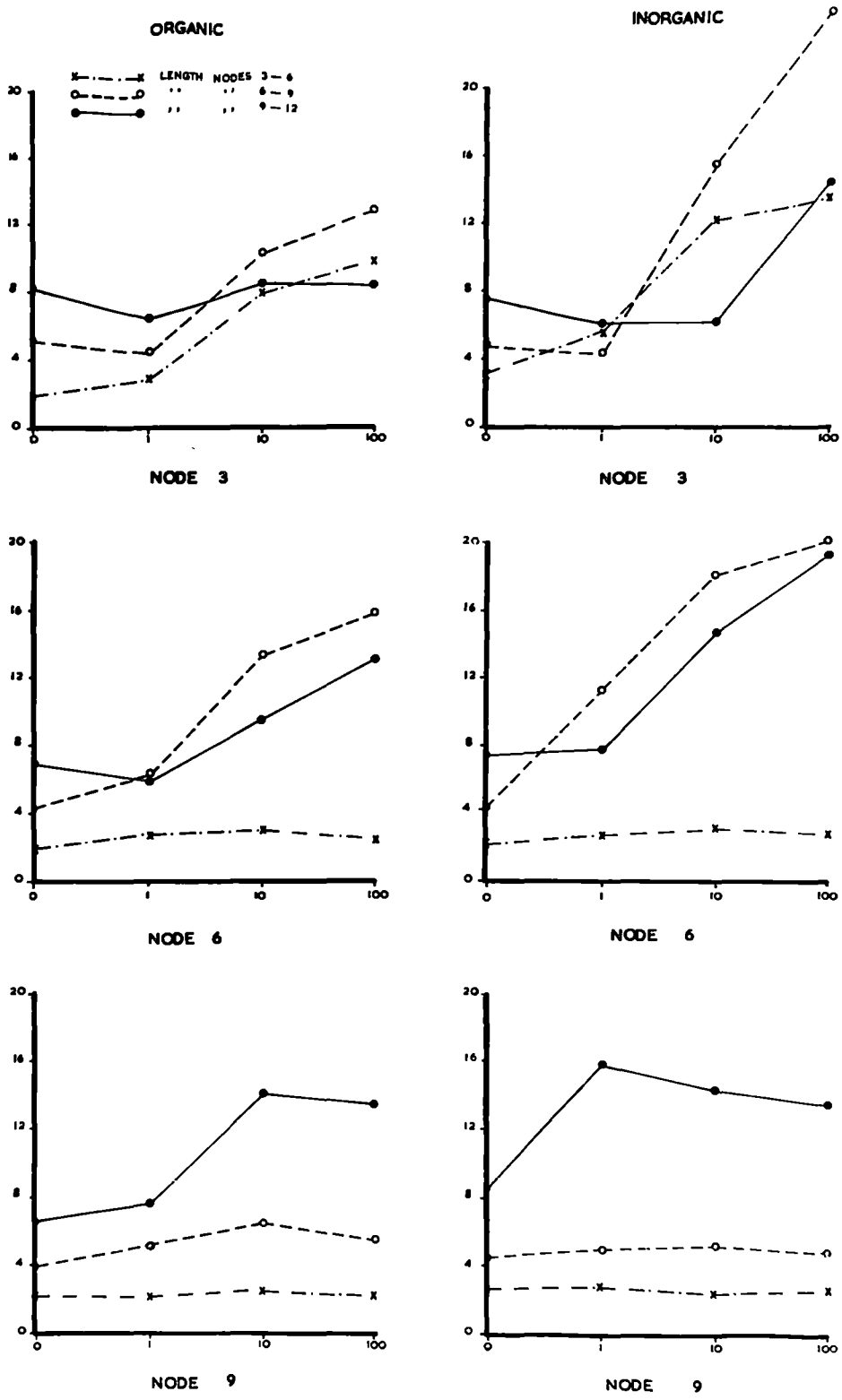


Fig 4.4. Effect of GA on length growth of Greenfeast plants grown under different nutrient conditions (organic or inorganic). Ordinates, GA dose µg: abscissae, length cm. The node numbers under the graphs indicate the nodes at which GA was applied.

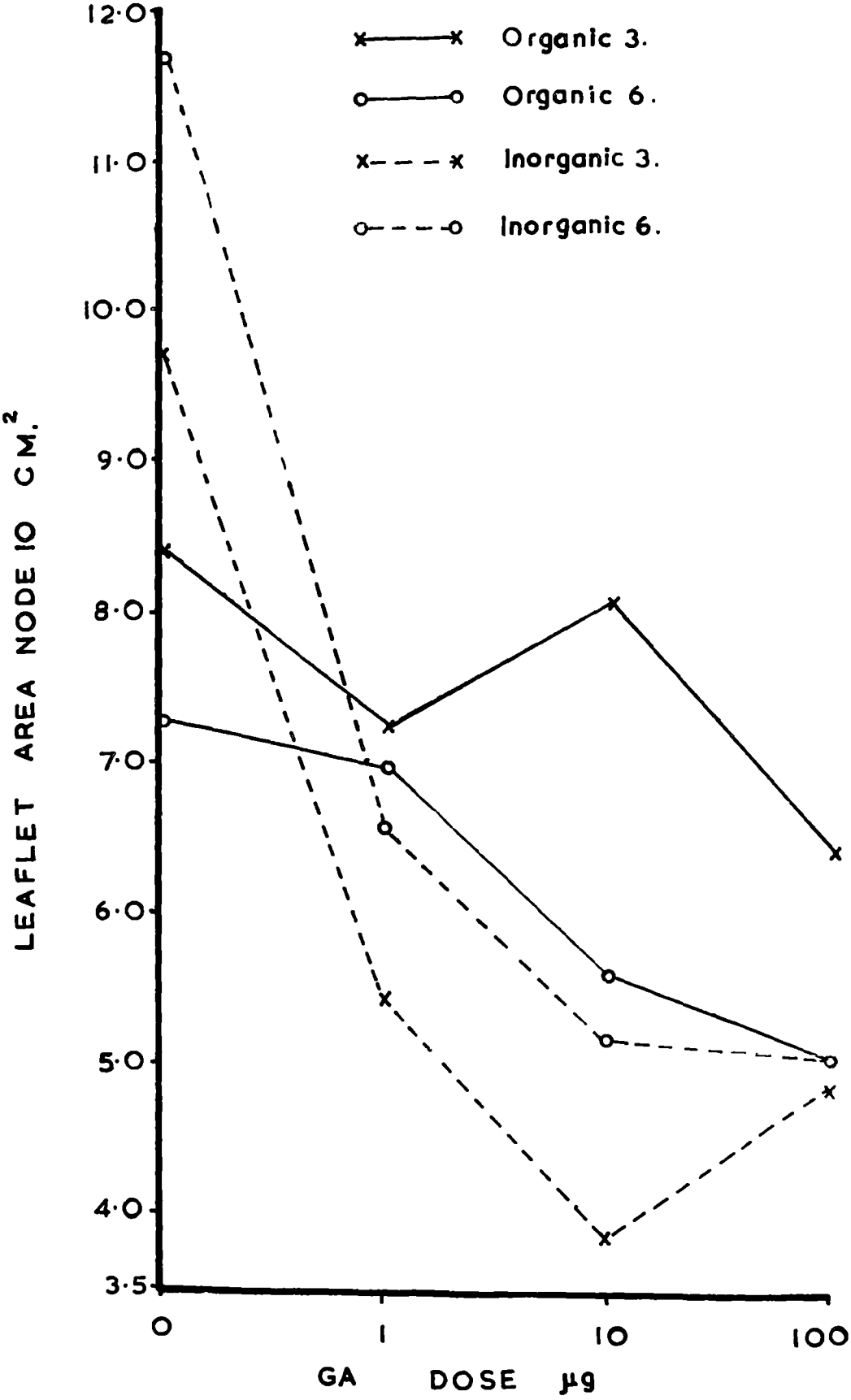


Fig. 4.5. Effect of GA and nutrient supply on leaf area in Greenfeast. GA applied at nodes 3 or 6. Data for node 9 applications not shown as there was no treatment effect.

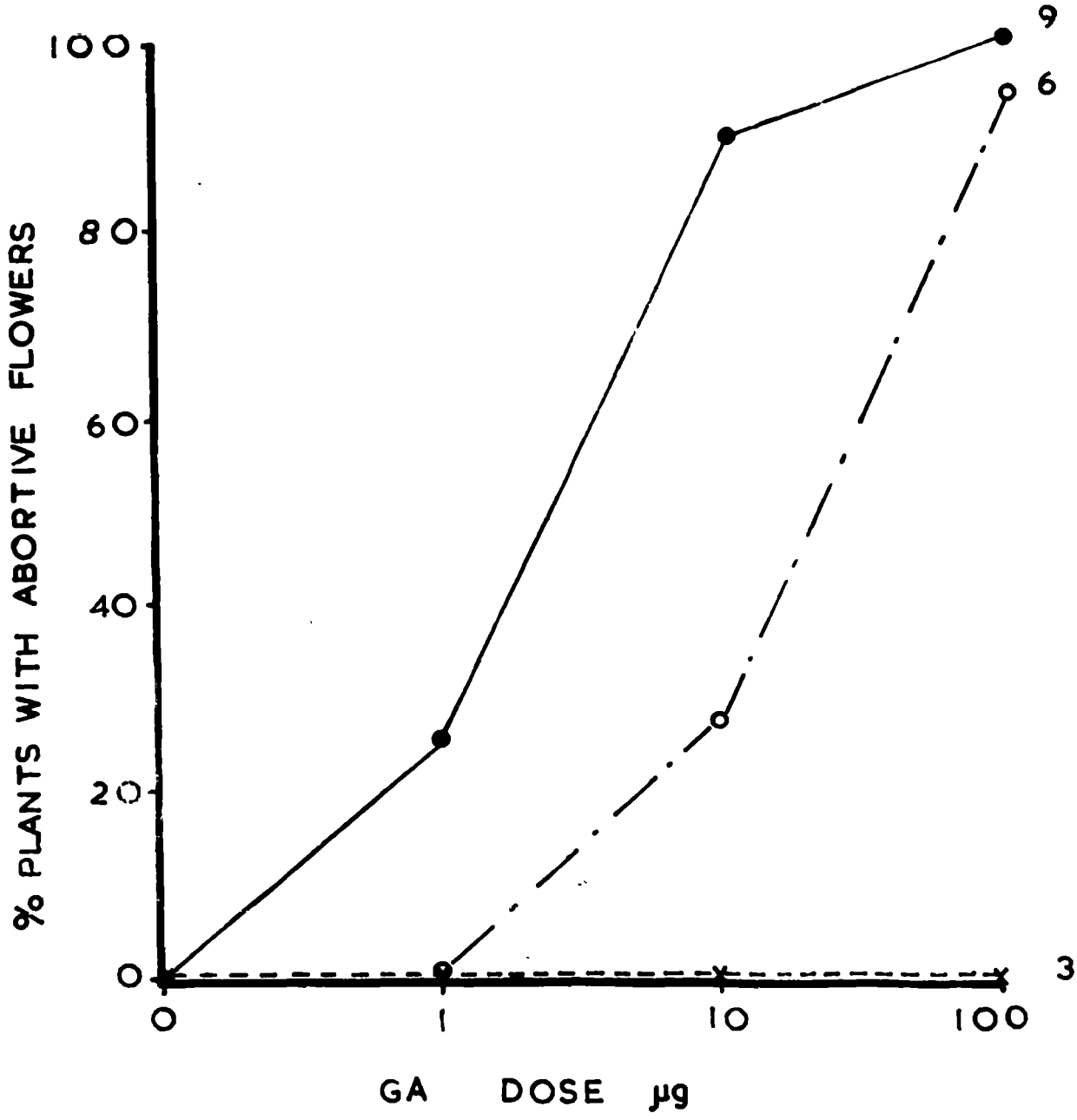


Fig. 4.6. Effect of GA on production of abortive flowers in Greenfeast. Data for inorganic nutrient treatments only. Numbers to the right of each curve indicate node at which GA was applied.

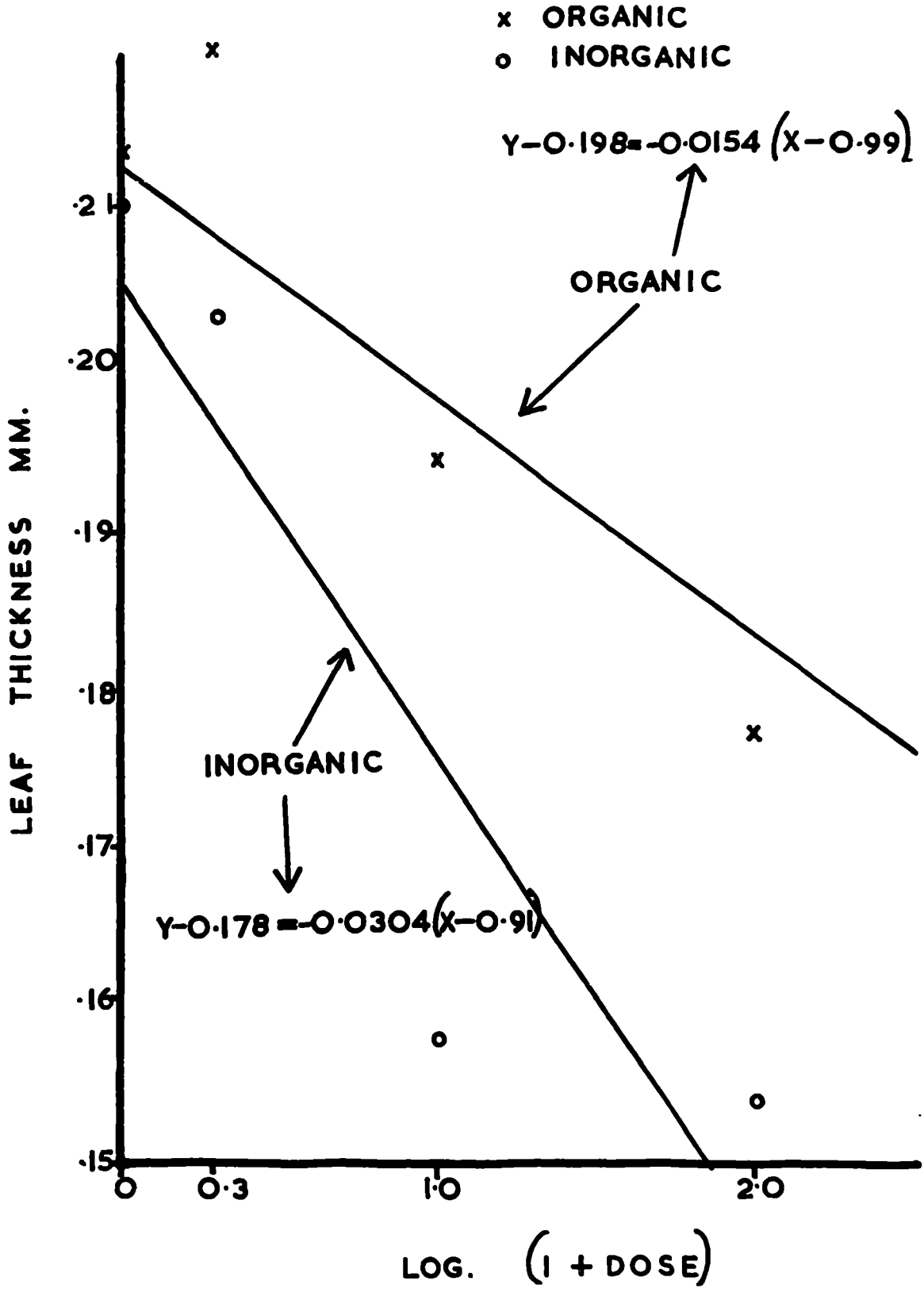


Fig. 4.7. Regression lines of leaf thickness at node 10 on log (1 + dose GA). Data only shown for applications at node 6.

vi. Comparison of GA effect with those produced by other substances.

Since the nature of the plant's response to GA was not completely known, an experiment was planned in an attempt to find possible interactions between GA and other chemical substances giving physiological responses. These substances were kinetin, indole-acetic acid, adenine sulphate and maleic hydrazide. All of these except kinetin have been used in other experiments described in this thesis. Kinetin (kindly supplied by Mr. T. Lewis, C.S.I.R.O. Hobart) was included because of its recently discovered growth promoting properties. An experiment using different concentrations of kinetin has since given no results but this may have been due to its instability. Since this work has been done Brian and Hemming (1957a, b and c) have published reports on interactions between maleic hydrazide, kinetin, cobaltous ions, acetate and GA. All these substances except maleic hydrazide were tested on stem sections. They have made the interesting suggestion that kinetin and IAA combine to give a growth inhibitor. This inhibitor may or may not be connected with the inhibitory system these workers proposed to explain the differences in response of intact plants and isolated internodes to GA (Brian and Hemming 1958). In their paper on cobalt and acetate they conclude that the effects of these substances, though superficially similar, are produced through different mechanisms.

Experiment 4.6. The experiment was restricted to the variety Greenfeast since sufficient glasshouse space was not available for a larger experiment. For the same reason, all possible combinations of the substances tested were not used, the substances only being used singly and in all combinations of pairs. The treatments given were thus as follows:-

Control (C)
Gibberellic Acid (GA)
Kinetin (K)
Indole acetic acid (IAA)
Adenine sulphate (AD)
Maleic hydrazide (MH)
GA + K
GA + IAA
GA + AD
GA + MH
K + IAA
K + AD
K + MH
IAA + AD
IAA + MH
AD + MH

All chemical substances were applied in alcohol/water solution to the leaf at node 3, since it was decided that soaking of cuttings in solutions was not suitable. 1 µg of each substance were used for each of 2 applications, i.e. 2 µg in all were applied. Plants were grown in natural daylight supplemented to give an 18 hr.photoperiod. They were scored for F, U, Ca, area of stipules (AS6), and leaflets (AL6) at node 6 and shoot lengths from nodes 6-10 (L6-10).

The results are summarized in table 4.12. By far the largest number of significant differences (from control) were obtained in treatments involving GA.

Discussion. GA caused a delay of about one node in F in all cases except where combined with MH. This is to be expected since MH has been shown by Paton (1956) to reduce F. In this case MH by itself had no effect. IAA * K caused a delay in F significant at the 1% level. IAA and K separately produced responses significant at the 5% level, so their effects may be additive. The effects of IAA and AD are for the moment unexplained, especially since Borgström (1939) showed that IAA is essential for flower formation in peas. It may be that the endogenous supply is optimal and added IAA thus has inhibitory effect.

The only treatments effecting U are those involving GA. This effect has been discussed previously in this chapter. The lack of effect on MH in reducing was unexpected, but may have been a result of too small a dose. It has been generally found that the control values in this experiment were rather low, presumably by chance, and thus only those differences significant at the 1% level have been considered.

The variance of Ca was smaller than usual for Greenfeast. Many of the treatments caused a significant delay in Ca, but those involving GA were by far the largest (about one nod

This is the first experiment in which GA has been found to increase photosynthetic area (in this case stipules and leaflets). The plants in this experiment grew very uniformly and were very healthy. Apart from this there was no obvious reason why the leaf area should have been increased by GA. The GA treated leaves did not show the usual yellower colour, but the GA effect on length was of the usual magnitude. It appears that some unknown factor must be limiting in those plants which show a decrease in leaf area when GA is applied. MH also increases leaf area in most of its combinations.

The only treatments which increase length are those in which GA is included. MH caused a slight reduction in the GA effect. This is to be expected from the results of Lukovac and Wittwer (1956) and Brian and Hemming (1957a).

Table 4.12

Effect of chemicals on growth of Greenfeast peas.

Data for node of first flower (F), first node with more than 2 leaflets (Ca), area of stipules (AS6) and leaflets (AL6) at node 6, and length between the nodes indicated.

Treatment	F	U	Ca	AS6	AL6	I_{2-6}	I_{6-10}
Control	15.22	11.86	11.86	2.59	3.63	4.42	8.58
GA	16.52	14.32	12.80	3.39	4.96	8.24	25.34
K	15.38	12.12	11.68	3.12	4.18	4.90	9.72
IAA	15.92	12.04	12.16	3.30	4.57	6.14	8.42
AD	15.80	12.36	12.28	2.75	3.72	4.78	9.34
MH	15.52	12.28	12.26	3.25	4.55	4.62	9.26
GA + K	16.12	14.32	12.24	3.36	5.02	8.86	25.56
GA + IAA	16.42	13.56	12.86	3.25	4.75	8.00	23.56
GA + AD	16.48	13.80	12.98	2.90	4.29	7.82	23.74
GA + MH	15.58	13.80	12.20	3.53	5.00	8.38	21.92
K + IAA	16.08	12.32	12.42	2.94	4.21	5.86	9.78
K + AD	15.52	12.20	12.22	2.83	3.78	4.62	9.62
K + MH	15.62	12.92	11.82	3.23	4.86	4.96	9.86
IAA + AD	15.56	12.28	11.82	3.13	4.16	5.06	9.52
IAA + MH	16.06	12.46	12.16	3.13	4.14	5.08	9.88
AD + MH	15.58	12.32	12.00	3.31	4.82	5.48	9.86
L.S.D. 5%	0.57	1.06	0.22	0.49	0.80	1.19	4.17
L.S.D. 1%	0.76	1.41	0.29	0.66	1.06	1.58	5.54

Figures underlined differ significantly from Control at the 1% level.

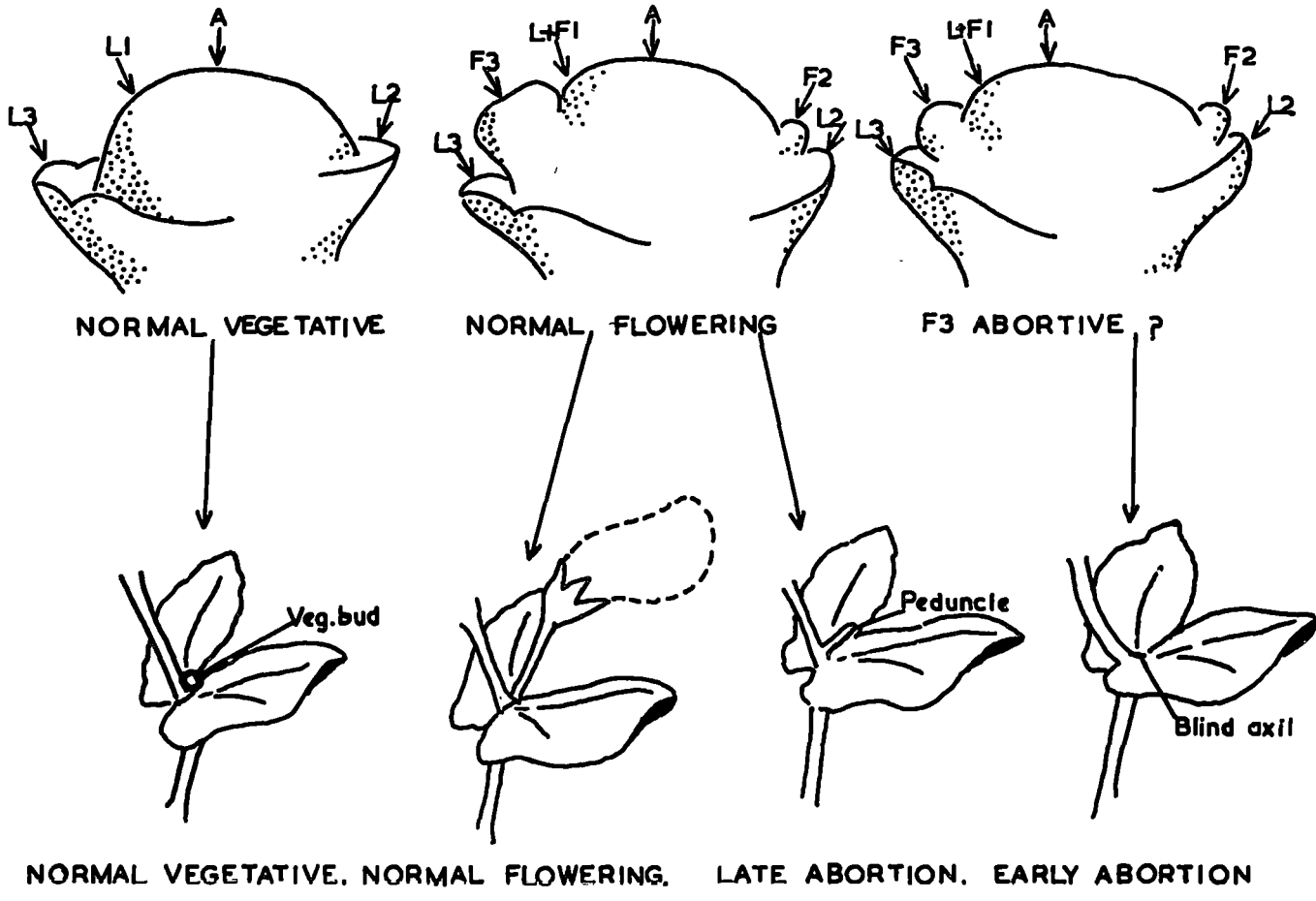
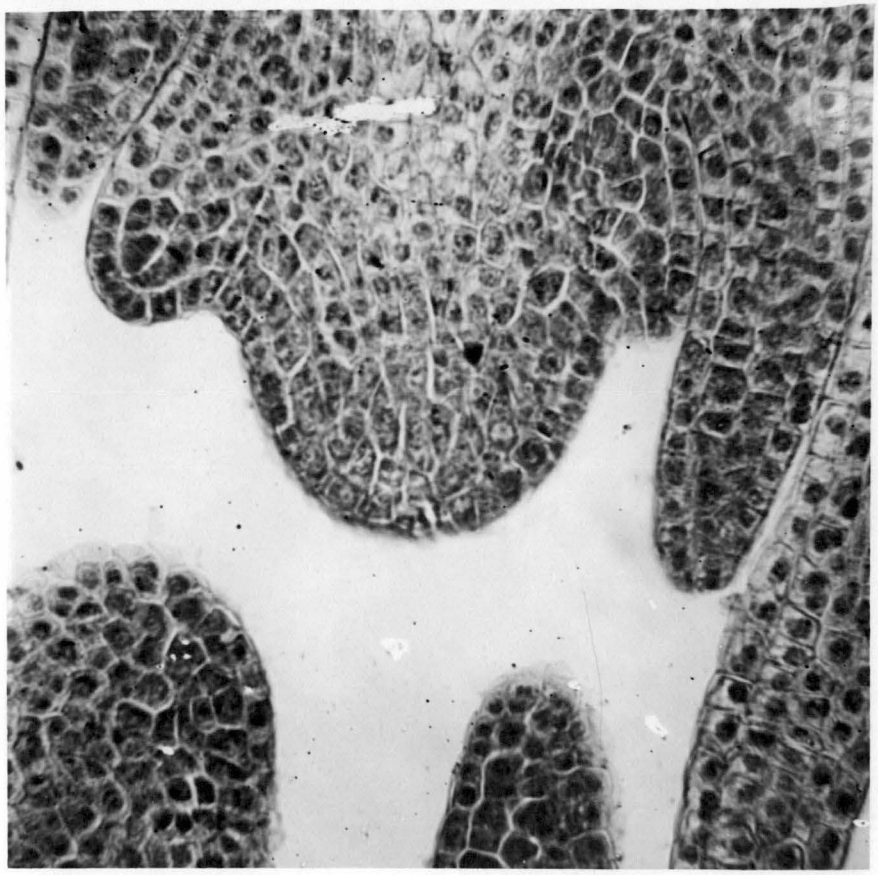
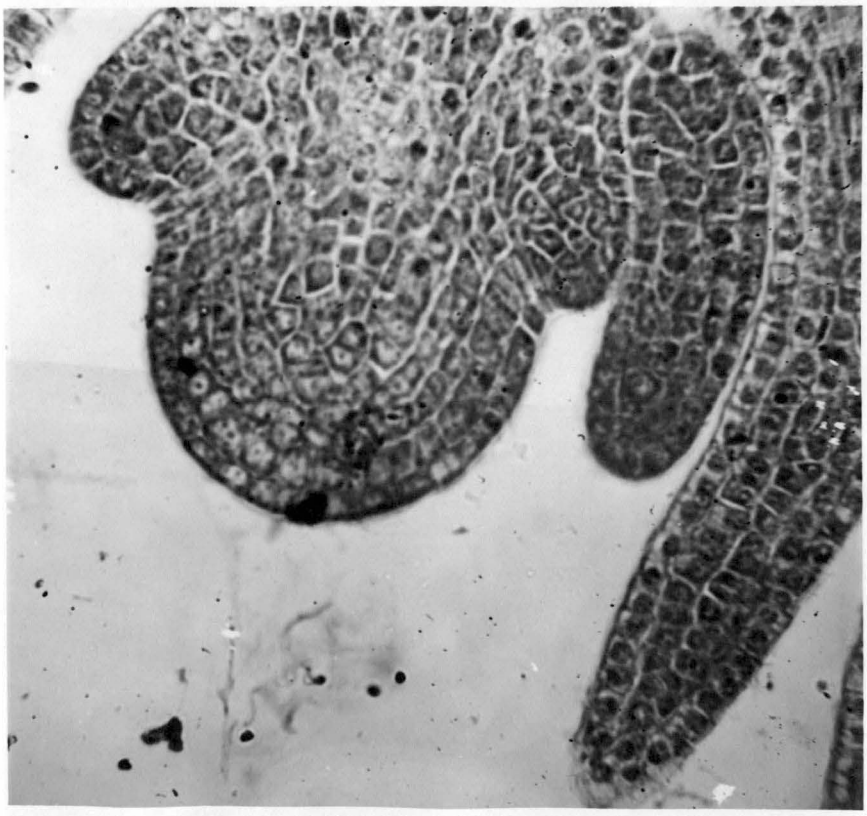


Fig. 4.8. Possible relationships between condition of apex and corresponding mature leaf axils.

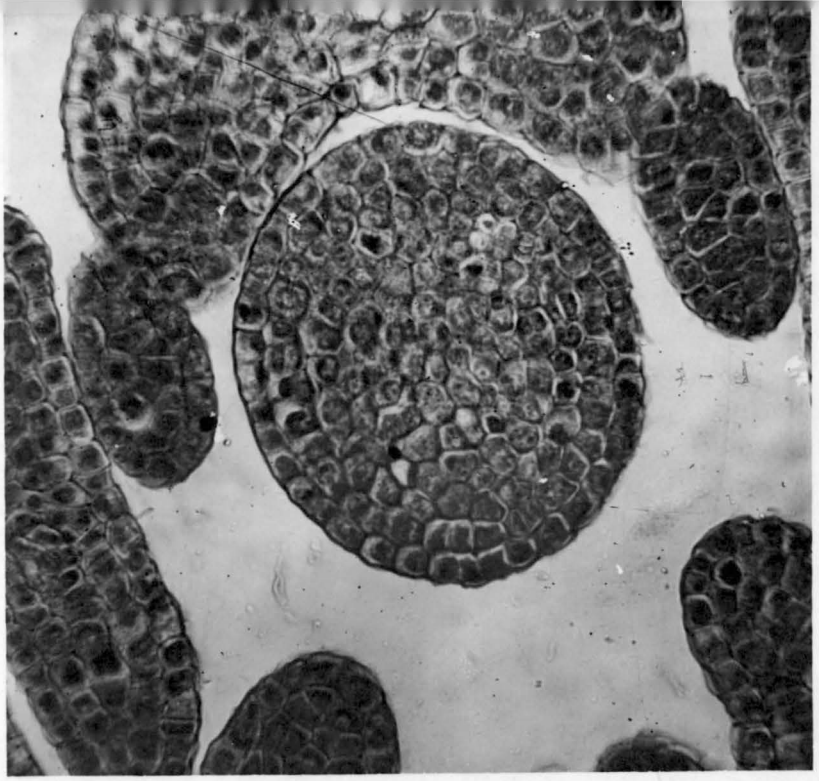


L.S. APEX TREATED WITH GIBBERELLIC ACID

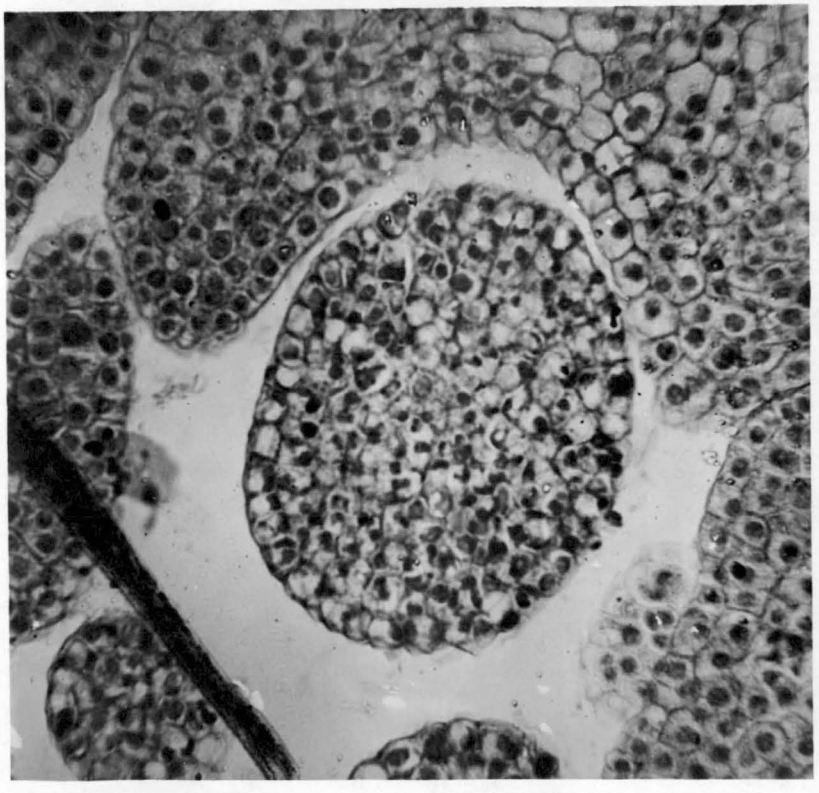


L.S. NORMAL APEX

Fig. 4.9. Longititudinal sections through Greenfeast apices to show effect of GA in disrupting the regular arrangement of cells. The tunica and corpus cannot be distinguished in treated sections.



T.S. NORMAL APEX



T.S. APEX TREATED WITH GIBBERELLIC ACID

Fig. 4.10 Transverse sections through apices corresponding to those shown in fig. 4.9. The GA treated apex is the least well organised. Prominent nuclei in GA treated apex are due to slightly different staining time.

CHAPTER 5.

GENERAL DISCUSSION

1. Flowering

Flowering in different species of plants has usually been measured quantitatively by one of three main methods. These are:

(a) Time between germination and some easily recognisable stage of flower formation (e.g. anthesis).

(b) Number of nodes between cotyledons and first flower.

(c) Number and/or state of development of flowers formed in a given time.

All three methods can be applied to both terminal and axillary inflorescences. The first two in particular can be adapted to measurement of flower initiation. In chapter 4, several experiments with GA have emphasised the differences which exist between flower initiation and mature flower production.

Many workers have suggested that a "minimum leaf number" must develop on a plant before flower initiation can occur. Holdsworth (1956) has criticised this concept under some circumstances, suggesting that certain combinations of chemical and physical treatments can reduce the minimum leaf number. However Holdsworth concedes that minimum leaf numbers can give a good indication of the condition of "ripeness to flower" in some plants (e.g. soybean. There is no doubt that minimum leaf numbers are a useful tool for discussion, although it is probably best as Anderson (1955) suggested (for terminal inflorescences) to restrict the term to plants growing under reasonably natural conditions. Each plant will then have a minimum leaf number which reflects its genetic constitution, but which requires a certain combination of environmental conditions for its expression. Ultimately, the genetic and environmental effects will be defined in terms of the biochemical and biophysical status of the plant's cells and the physiologist will then be able to culture flowers *in vitro* from undifferentiated plant tissue. The achievements of workers on tissue culture is steadily increasing. For example,

Skoog and Miller (1957 etc.) have cultured various organs from tobacco callus; de Capite (1955) cultured fruits from flowers and Kawata (1957) has obtained flowers on plants cultured from apical domes of rice and wheat. Another important, though more specialized report is that of Vasil (1957) who cultured anthers of Allium cepa. By the use of appropriate mixtures of GA and kinetin, Vasil succeeded in obtaining tetrads from anthers excised at leptotene. This represents a considerable advance over earlier cultures of anthers and shows that GA and kinetin are vital to the fundamental processes of the plant cell.

Minimum leaf numbers are a good measure of the physiological potentialities of different plants. They can easily be equated with the node of first flower, (F) which under optimal conditions is equal to one plus the minimum leaf number. In peas, F varies widely between varieties, the lowest value known at the moment being that of an "acacia" line which commences flower initiation at node six. Since there are usually about six nodes present in the embryo prior to germination, it seems that no leaves are necessary for production of flower initials in this variety. It is possible that in this line, flowers can be initiated while the young seed is still in organic contact with the parent plant. The minimum leaf number for such varieties may simply be equal to the number of nodes present in the embryo.

Several early (sn) pea varieties can initiate flowers without light (Borgström 1939, Leopold 1949). There is a marked difference in some varieties between the ability to initiate and the ability to develop flowers. This is clearly shown in the "acacia" line in which functional flowers are not produced until two or three nodes after flower initiation. Each initial gives rise to successively larger buds until a mature flower is formed. All the earlier buds abort and abcise. Thus it appears that leaves and/or light are necessary for the development of mature flowers. Vince (1956) found that longer wavelengths of light were more effective than short in promoting flower development in early peas. This suggests that a photoreaction is necessary for the production of

mature flowers. The nature of the reaction is unknown, but an interesting possibility has been raised by the report of Hadan (1956) that in Kalanchöe larger quantities than normal of various amino acids (e.g. asparagine, cysteine, histidine and aminobutyric acid) were produced in the induction period. Hadan did not correlate these substances with any particular phase of flowering (e.g. initiation or development).

The need for distinguishing between flower initiation and development was stressed by Purvis (1934). Recent evidence has suggested that the photoperiodic responses in some plants may be partially restricted to flower development. For example, de Zeeuw (1957) in a very interesting preliminary report showed that Xanthium (normally considered an obligate short day plant) can initiate flowers in long days under certain temperature regimes. These initials normally remain dormant. Similar results were described by Haupt (1954) who obtained flower initials on a short day plant grown in continuous light, but these initials did not develop. In experiments described in this thesis, GA has been found to affect both flower initiation and development. In experiments without GA, the first flower produced has almost always developed normally. This indicates that the treatments applied affect both flower initiation and development to the same extent, or alternatively they have affected only flower initiation (when the treatments cause flowers to be produced at a higher node than normal).

It is evident that the results obtained by treating peas with GA vary greatly between varieties. This has already been stressed for increases in length (Brian and Hemming 1955) where dwarf varieties respond greatly and tall varieties slightly, if at all. Varietal differences also occur in flowering effects. These may partly explain why other workers have found no effect of GA on node number of first flower (Bukovac and Wittwer 1956) or a slight reduction in days to flower (Brian 1957). Among the varieties used in this laboratory, the response of flowering to GA has varied. In the variety Alaska, GA has little or no effect on flower initiation but impedes the production of mature flowers. The latter is probably the major effect. It is not known at present how many flowers are caused to abort by GA at an early stage in their development (Fig. 4.8

Early dwarf and late tall varieties have been used by most workers who have performed experiments with GA on peas. In the work described here, an attempt has been made to link the results obtained with the genetic constitution of the plants. Thus all combinations of early and late plants with tallness and dwarfness were used. When GA is applied to the leaf at node three, it is usually too late to effect flower initiation in early peas. This could explain why Brian (1957) obtained only a slight accelerating effect of GA on the variety Meteor. GA can increase the rate of node expansion (see Chapter 4) and thus applications of GA after flower initiation could result in earlier flowering as measured by days to the appearance of flowers. Late tall varieties show little effect of GA on flowering. Thus the failure of other workers to obtain an effect of GA on flowering in peas can be fairly readily explained. The lack of effect of GA on late tall varieties may be because most of the GA has been metabolised by the time flower initiation occurs (see Chapter 4). However, there can be an effect of GA under certain conditions, for example in unvernalised Telephone plants, especially under short day conditions. The most consistent delay in position of node of first flower in GA treated plants has been with the *le* varieties Greenfeast and Massey (with seed treatment). The possible reason for the smaller response of the *le* plants has been discussed in Chapter 4. Greenfeast, being a late dwarf variety, responds readily to GA in all respects when treated either on the seed or on the first true leaf. This response is possibly connected with the effect of GA on internode elongation, comparable with the effect of the *La* gene on flowering (Zarber 1958). Data from the F_2 generation of crosses *le* x *le* suggest that plants with the dominant *La* flower about half a node later than plants with the recessive *le*. The effect of GA on peas is greater than that of the *La* gene (Chapter 4) on both flowering and internode extension. This suggests that GA and *La* act through the same mechanism, thus supporting Lang's (1957) proposal that the effect of GA on flowering may be entirely secondary to its effect on shoot height. This proposal was based largely on work with rosette plants in which GA stimulates flowering, but seems to hold also for plants in which GA delays flowering (e.g. *Enlanchhe* and peas).

The apparent correlation between flowering and the number of expanded nodes (E)

in GA treated plants may be misleading, as the effect of GA on E may occur after flower initiation. The number of expanded nodes and the node of first flower are highly correlated under certain conditions, such as poor food supply, which lowers both F and E (Holdsworth 1958). The same conditions may increase the time to flower initiation. Holdsworth found that F and E were not always correlated in Impatiens, and Wittwer and Teubner (1957) found that high nitrogen treatments on tomato resulted in earlier flowering. In the only nutrition experiment described in this thesis, plants grown under reduced nutritional conditions showed a highly significant reduction in node of first flower, but no effect on the number of expanded nodes. In the discussion of this experiment, it was suggested that certain mineral elements may have been responsible indirectly for the results obtained, by affecting the metabolic rate in certain plant organs (e.g. leaves). Whatever these factors were, they did not affect node formation. This type of experiment may prove useful as a means of separating the two processes (flowering and node formation) in photoperiodically indeterminate and facultative long or short day plants.

Further evidence against the hypothesis that a lower flowering node can be explained solely in terms of vegetative growth is given in the experiments on cuttings. In these experiments, treatments lowering the node of first flower sometimes increase (or have no effect upon) the number of expanded nodes. Barber (1958) has shown that initiation of the first flower in peas does not inevitably lead to the end of the vegetative period (as it does in most plants with terminal inflorescences), since under certain combinations of environmental treatments reversion from flowering to vegetative growth can occur. Such reversion has also been found in Fuchsia fruticosa by Shimoda (1951).

The apparent conflict between vegetative and reproductive growth in plants has been interpreted in terms of inhibitory and promoting substances. These are usually referred to flowering, although Guttridge (1956) has proposed a photoperiodically controlled vegetative growth stimulus in strawberries. Several workers have postulated a flower inhibitor (colysanthin) to be produced in unfavourable photoperiods (e.g. Schmitz 1956

and Harder 1948 for Kalanchöe). Others postulate inhibitors in young, growing leaves (e.g. Bünning and Kondor 1954). This last type of inhibitor can often be equated with a vegetative growth promoting substance. De Zeeuw (1956) suggested that the inhibitory action of young tomato leaves may be explained (a) by the production of a colysanthin: or (b) by competition for available auxin between leaves and flowers. Fisher (1955) also suggests that auxin may be involved and proposes a balance between young leaves (inhibitory) producing auxin and mature leaves (stimulatory) producing florigen. In an interesting paper, Kinney and Hamner (1957) who worked with soybeans, postulated an endogenous rhythm (on a 24 hour cycle) which is inhibitory to flowering. This acts in opposition to a process producing florigen. Cool temperatures reduce the effect of the endogenous rhythm. Downs (1956) and Cathey and Borthwick (1957) have both suggested that under repeated cycles of red and far-red light, the red light builds up an environment which is inhibitory to flowering. Thus it appears that flower inhibitors may be fairly widely distributed in plants. It is most likely that in peas and other plants a balance between flower promoting and flower inhibiting substances finally governs the production of flowers.

The colysanthin in late pea varieties seems not to be connected with auxin metabolism (Paton 1956). It is, however, produced in unfavourable photoperiods (i.e. short days). The chemical nature of the inhibitor is unknown, although it may be partly contained in yeast extract, since Haupt (1957a) succeeded in making early peas photoperiodic when grown on a medium containing yeast. Experiments in this department are aiming to purify colysanthin from yeast extract or leachates (see chapter 3).

Figure 5.1 gives a diagrammatic representation of flowering in peas, and attempts to correlate the work of Harder, Haupt, Paton and Sprent. The scheme is confined to the effects of the major locus Sn. Other possible loci have been discussed by Harder (1958). Harder's terminology has been followed: π represents precursors, ϕ represents florigen and K represents colysanthin.

The reaction $K \longrightarrow \phi$ has been discussed by Paton (1956). Many of the results obtained with late pea varieties can be explained equally well as either (a) reaction $K \longrightarrow \phi$, or (b) an inactivation of K. Paton (1956) favours the first alternative.

If the two factors are interconvertible, as suggested by Paton on physiological grounds and Harber on both genetical and physiological grounds, then Sn plants will have produced their K from ϕ. This, as Harber has stated, immediately suggests that sn plants are lacking in the ability to convert ϕ to K, a common type of metabolic "fault" in recessives. The reaction K ——— ϕ must take place at a relatively slow speed, accelerated by both vernalization and long day treatments. Even when the seedlings are strongly vernalised and grown in long photoperiods, they are unable to cope with all the K from the cotyledons, and some passes to the apex to exert its effect. Some of this residual K can be removed by leaching (see chapter 3). The promotion of flowering by leaching in water can be considered a "negative" effect, i.e. merely a removal of inhibitor. On the other hand, the flower promotion by vernalization and long days is a "positive" phenomenon resulting in the formation of florigen (ϕ). All three treatments (vernalization, long days and leaching) can act on flowering at the same time, as has been shown in chapter 3. The action of short days on Sn plants is to stimulate further production of K, probably in the leaves (Paton 1956). In long photoperiods this K may be converted directly into ϕ, possibly explaining the promoting effects obtained by Laupt(1957b) for mature leafy interstocks grafted with early scions.

The reverse reaction ϕ ——— K does not normally occur in sn plants. Recent work by Laupt (1957a) suggests that the missing factor in these plants can be supplied by yeast extract. sn plants grown by Laupt on this substrate responded to photoperiod. The factor supplied by yeast cannot be colysanthin itself, since on the system outlined above colysanthin is formed from ϕ. sn plants contain ϕ and thus the effect of yeast extract must be to provide some accessory growth substance (or co-enzyme). The reaction giving rise to K only takes place in short days and probably in the leaves (as in Sn plants). Long photoperiods may inactivate the K once more. The conversion of K into ϕ must be light dependent, since Laupt's "embryos" flowered at almost the same node in darkness and in long days. When all the K has been used up in the apex or converted

into ϕ, then flower initiation can take place.

The light requirement for flower initiation in peas is secondary, being only necessary in plants containing K. In some peas (e.g. "Acmeia") all the substances necessary for flower initiation are present in the cotyledons. In others (e.g. Massey and Alaska) they are formed very soon after germination. In these varieties a minimum leaf number has to be laid down in order for flower initiation to occur. This minimum leaf number reflects the time taken for one or more substances to be produced or mobilised to complete the requirements for flower initiation. It does not represent a light dependent process.

The processes of flower development require leaves and light for their completion. The manufacture of the substances required can take place before or after flower initiation according to the variety. In late varieties, flower development proceeds to completion as soon as the first flower is initiated. This is presumably because all the substances requiring a light reaction have been synthesised by the time initiation takes place.

That GA does not affect flowering in peas through the inhibitor system seems fairly certain, because there is very little interaction between GA and photoperiod. Whether or not GA has an effect interacting with vernalization is not yet certain. Although this appears to be the case from the experiments described here, it is possible that some of the GA diffused away from the seedlings before it had time to have an effect. An exact interpretation of the effects of GA on flowering in peas is not easily made, as there are so many side effects of GA. Since GA effects both flower initiation and development it is possible that the two effects take place in the same way. It was suggested in chapter 4 that this could be by means of apical disorganisation.

In outlining the above theory, care has been taken to avoid implying that specific substances are required for any one stage in flowering. Skoog and Miller (1957) have suggested that the search for specific factors influencing development may have been partially fruitless because it is really a balance of factors that is required. This

seems a reasonable argument. There is no doubt that certain specific substances such as GA and IAA may have a very great effect upon physiological processes. However, their effect may be modified according to the internal state of the plants at the time of application. If a complex balance of substances is required, it may be that different components of this balance may become limiting under different conditions. This could easily account for isolated reports of the effects of single substances on processes such as flowering. For example, Saubert von Euson (1948) found that ascorbic acid was necessary for the production of mature flowers in her pea cultures. Under other circumstances this substance may not be limiting.

No attempt has been made in this discussion to deal in detail with photoperiodic flowering phenomena in peas. These have already been dealt with by Paton (1956) and Barber (1958).

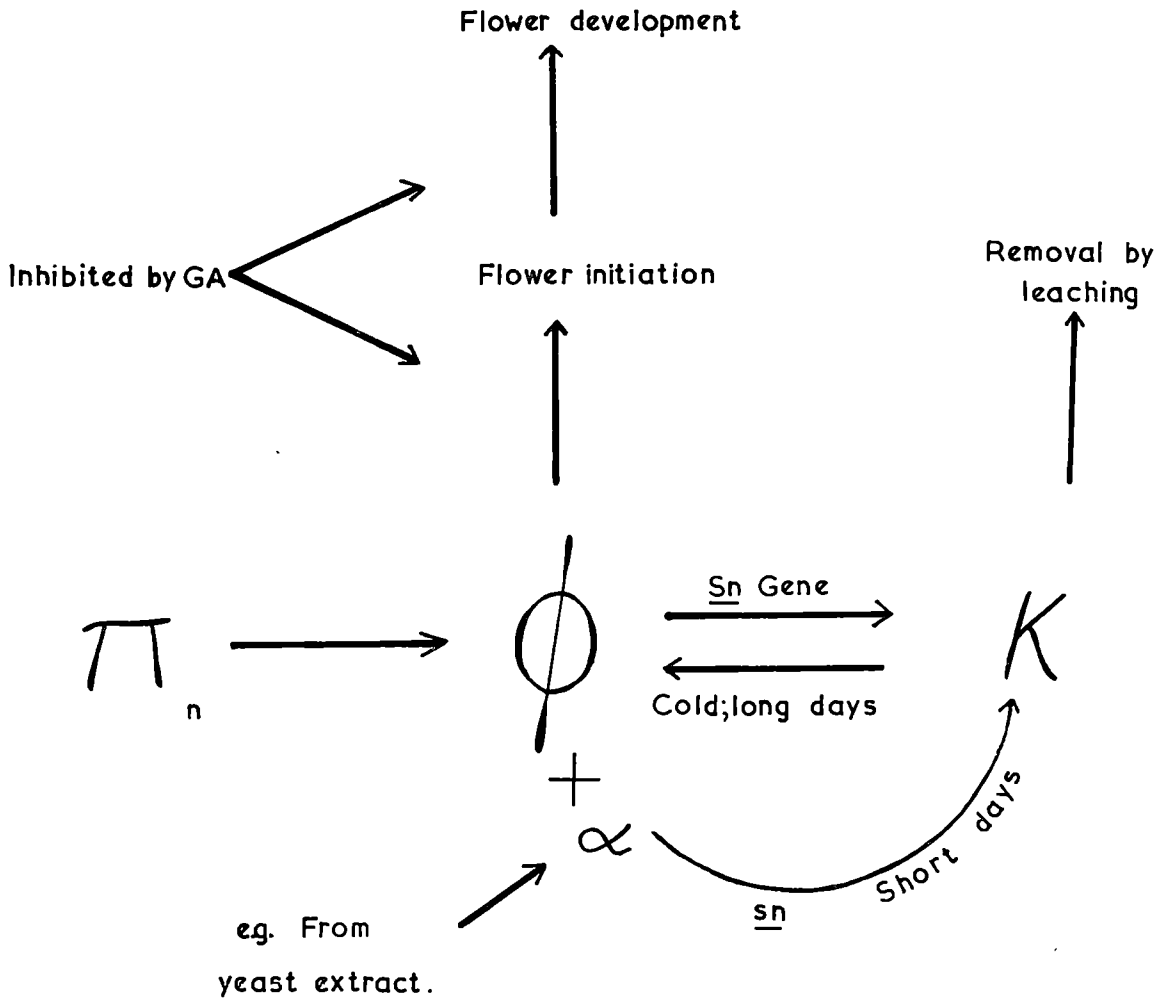


Fig. 5.1. Diagram to show the possible pathways leading to flowering in peas.

ii. Leaf Growth

Leaf growth is usually measured as leaf area, which is vital to the photosynthetic processes in plants. Gregory (1926) working on Cucumis showed that the rate of growth of leaves proceeds in a manner suggesting an autocatalytic reaction. He described the effects of temperature and nutrient supply on leaf area. Leaf area in peas is probably governed by a very similar system. In this thesis leaf area measurements are confined to mature leaves. Measurements were made in quantity only in the later experiments, after the photo-electric measuring system had been devised.

The genetics of leaf area production in the pea varieties used are completely unknown. It is obvious from table 4.9 that varietal differences do exist. It is known that temperature exerts a major effect on leaf area (Paton unpub.) and that photoperiod can also have a marked effect (Paton 1956). The photoperiodic effect is accentuated as the plant gets older, which may account for the non-significant effect obtained in experiment 4.2 for leaflet area at node 6 (data not given). GA usually decreases leaf area, but the effect varies with nutrients supplied.

Total leaf area at any node in peas is the sum of the area of a number of parts, i.e. stipules and leaflets. For the purpose of simplification, stipules will be largely omitted from this discussion. It was shown in Chapter 2 that there is a limiting area for the two basal leaflets (which will be termed leaflets 1 and 2). Any increase in leaflet area beyond this limit at a particular node is shown by an increase in leaflet number. The maximum area for leaflets 1 and 2 can only be reached in non-limiting nutrient supplies. The factors governing maximum area of leaflets are not known, but could well be something fairly simple such as efficiency of the vascular supply. This was found by White (1956) to limit the size of runner bean leaves.

The relationship between leaf area and leaflet number is more complex than it seems at first. Additional leaflets are not, as it first appears, only formed when the areas of leaflets 1 and 2 have reached their limits. Leaflets 3 and 4 are initiated well before division of cells forming the lamina of leaflets 1 and 2 is complete (assuming leaflet histogenesis in peas to be similar to that described by Foster (1935) for Carya).

Expansion of leaf lamina can be influenced when the developing leaves are macroscopically visible (Horton and Watson 1948). The correlation between limiting area of leaflets 1 and 2 and production of more than 2 leaflets may therefore by misleading. It can, however, be fairly readily explained. If, for example, the first node with more than two leaflets is 14, then these leaflets would be still capable of being influenced when about 11 nodes are expanded. Food supplies at this stage of the plant's growth are likely to be plentiful and they would naturally be more readily available to leaflets 1 and 2, being nearer to the main axis. Thus these leaflets are very likely to attain their maximum area. For this reason leaflet number can be retained as a measure of leaf area in normally growing plants.

It is not impossible that the node at which more than two leaflets are produced is related to the leaf area at the time of initiation of this node. The relationship however is complex and it has not been found possible to equate the two factors numerically. It has been suggested in chapter 3 of this thesis that the leaflet number in peas is limited by the concentration of "lobing" substance similar to that described by Ujohu (1956) for Ipomoea. The situation in peas is a special case of heteroblastic development as found in Ipomoea where older leaves are more deeply lobed than younger ones. In peas, lobing is complete, leading to the formation of a definite number of leaflets. Allsopp (1954) has attempted to explain heteroblastic development in a variety of plants (including ferns) as being proportional to the size of the apical dome. Much of his data is convincing, but this situation seems unlikely to hold true for peas since the growth of leaflets 3 and 4 takes place away from the contact with the apex. It has been shown (chapter 3) that the supply of "lobing" substance to the apex can be altered by varying the rate of node formation. This conclusion has also been reached by Ujohu in his most recent paper on Ipomoea (1957). The anomalous behaviour of some cuttings can be satisfactorily explained on the basis of reduced rate of node formation. A similar explanation can be suggested for the production of 3 or 4 leaflets at node 4 in about one third of Greenfeast plants. A large amount of "lobing" substance may be

released as soon as the seed becomes fully turgid but before new nodes are formed. The leaflets at node 4 are very likely to be affected by such a release.

If leaflet number is governed by a "lobing" substance, then this substance or its precursors must be present in the cotyledons. This is clearly shown in experiment 2.1 and figure 2.1, in which cotyledons were removed. Removal of cotyledons also has the effect of reducing the rate of node formation which would tend to make greater than 2 leaflet leaves be formed at a lower node. However, the same treatment removes the necessary growth factor and thus leaves with 3 or 4 leaflets cannot be produced until further supplies of "lobing" substance are synthesised. The place of synthesis is almost certainly the leaves. For this reason leaf area over a limited range prior to initiation of nodes with more than 2 leaflets may be important.

In a small number of experiments leaflets thickness was measured. Variations in nutrient supply (or effective nutrient supply in the case of GA treated plants) probably explain most of the differences obtained.

From the data given in this thesis it appears that three separate factors or group of factors may govern leaf development. These are:-

(a) Nutrient supplies. These govern leaf area (and thickness) according to the system outlined by Gregory (1926).

(b) Factors limiting maximum area for any one leaflet. The vascular system may be a major limiting factor.

(c) A "lobing" substance which governs the number of leaflets laid down. Optimal conditions of other factors (e.g. food supplies) are probably necessary for full expression of the "lobing" substances. Production of "lobing" substance may be proportional to leaf area present at the time of initiation of nodes with more than two leaflets. Its effect is inversely proportional to the rate of node formation.

The size of any particular leaflet may be limited by (a) or (b) according to the environment under which the plant is grown.

iii. Stem Growth.

Brian and Hemming (1958) have recently proposed a three factor system which governs stem growth in peas. The factors are, (1) natural gibberellin-type substance; (2) auxin and (3) an inhibitory system. In a series of very interesting experiments, these workers correlated the differences in GA response of isolated and intact internodes of dwarf peas. Most of the work described in this thesis supports the conclusions of Brian and Hemming. Vlitos and Meudt (1957), working with the tall variety Alaska obtained very similar results. These workers found that GA can overcome the inhibition of internode expansion which occurs after the seedlings have been exposed to red, blue or green light. In addition, they confirmed the vital role of the shoot apex in the elongation processes. Although the work of Vlitos and Meudt is not yet as complete as that of Brian and Hemming, it does suggest that the processes of internode elongation in tall and dwarf varieties of peas are very similar. The work described in chapter three showed that tall (*Le*) varieties of peas are able to respond to GA by increasing in length, especially in the lower internodes. Such increases in length were also found by Vlitos and Meudt. If the effect of GA is to inactivate a substance inhibitory to internode elongation, then some of this inhibitor must be present in tall varieties. It is presumably lacking in "slender" plants. Thus if the effect of GA is linked with the action of the *Le* gene, plants with the dominant *Le* must have a smaller amount of inhibitor than those with *le*. It is usual for recessives to be lacking in the ability to perform one metabolic step. For this reason, it is more likely that the plants with *le* convert their growth inhibitor into either a growth promoter or else an inactive substance, than that they are unable to produce as much inhibitor as the dwarf varieties. A more complete investigation of the differences between the different known genotypes is highly desirable to find out whether or not the effect of GA on internode length is through both the *Le* and the *Cy* loci or if it confined to one or the other of them. In addition, it would be of interest to perform tests on the locus *Lm* (Lindqvist 1951) to see if the recessive *lm lm* ("micro") responds to GA. The system of length inhibitors governed by the *Cy* locus was originally proposed by Lamm (1937). He did not consider

the effects of the *La* locus, no reference being found in his papers to the genotype *La La Cy*$_1$ *Cy*$_1$ *Cy*$_2$ *Cy*$_2$. Lamm also states that "the fourth dwarf type *Cy*$_1$ *Cy*$_1$ *Cy*$_2$ *Cy*$_2$ was not found and seems to be rare". If the combination of the double *Cy* dominants is indeed rare, then it is most likely that the commercial varieties have one dominant and one recessive pair of *Cy* alleles. In any case they would not bear the double recessive, since these genotypes are easily recognisable. Thus, on the basis of their *Cy* complement alone, all commercial varieties of peas may be expected to respond to GA by increasing in length. The dominant *La* gene partially releases the *Cy* inhibition, possibly by means of a natural gibberellin. However, the normal *La* plants are much stronger than either the "slender" or GA treated plants and they do not have associated differences such as smaller leaflets. In addition the differences between the tall (*La*) and dwarf (*la*) plants can be largely (80%) accounted for by increased cell numbers (estimates based on measurements of the epidermis). The remaining 20% difference is due to increased cell length (Paton and Sprent unpub.). This is most likely to be due to an increase in the time over which cell division takes place rather than a marked difference in the rate of mitosis. The difference between GA and control plants is largely one of cell elongation, increased cell division being only just significantly greater in the GA treated plants (Sprent and class unpub.). This suggests that the effect of GA is to neutralise the *Cy* inhibition and thus to simulate "slender" plants rather than normal (*La*) talls. However, the results of the GA treatments in experiment 4.2 were most easily explained as an effect on the *La* locus. Unfortunately, the *Cy* complement of the commercial varieties is not known. With the evidence available, it is best to assume that GA can act on both the *Cy* and the *La* loci, but not necessarily in the same manner. For example, the *La* gene may act through a different natural gibberellin to the *Cy* loci, or it may act in conjunction with a naturally occurring cell division factor. The action of GA varies between species, causing mostly cell elongation in dwarf plants, but increasing cell division in rosette plants. Also, it is possible that if one gibberellin (GA) can cause different effects on different species, then different gibberellins (natural and GA) may have different effects on the same speci

Brian and Hemming's paper satisfactorily explains the effect of GA on dwarf peas. However, even under optimum conditions of GA and IAA concentration, internodes do not go on extending indefinitely. The final limiting factor is probably the onset of cell differentiation. In chapter 4, it was suggested that GA can upset the nature of the cell wall (figures 4.9 and 4.10). It may be that GA interferes with the processes of differentiation in the cell wall, thus enabling the wall to elongate for a longer period. If this were true then the inhibitor proposed by Brian and Hemming could act as a stimulator of cell wall differentiation.

iv. The Balance of Growth

Plant growth is a complex balance of factors which interact to varying extents, making it essential to interpret the effect of experimental treatments on one aspect of growth, in the light of concomitant effects on other aspects. The two major components of this balance are vegetative and reproductive growth. These components may be in apparent opposition. Brian and Hemming (1958) in their discussion of length growth in peas suggest that their three factor system (GA, auxin and inhibitor) for length growth may also govern other aspects of growth. The proportion of these three factors need not be the same for all aspects or all plants. The suggestion is based on the wide range of plants which respond to GA (see Stowe and Yamaki 1957). It is worth further consideration in the light of several interesting facts.

The effects of GA on different plant species vary greatly, particularly as regards flowering (Wurth et al. 1956). GA can promote or inhibit flowering in both long and short day plants and it can replace or not replace the requirement for vernalisation. In Hyoscyamus (Lang 1956), GA can replace the requirement for vernalisation. It also causes great elongation of the stem, transforming the plant from a rosette to a cauline habit. Lang (1957) has suggested that the effect of GA on flowering on this and on other species may be a secondary effect dependent of the effect of GA on internode expansion. This does not always appear to be the case, since in cabbages, GA induces bolting but without flowering. In this case GA does not replace the vernalization requirement (Wittwer et al. 1957). The general evidence indicates that GA probably does not act directly on flowering by the removal of an inhibitor. In most plants which respond to vernalisation, a substance "vernalin" has been proposed. This substance promotes flowering. In peas, where vernalisation has been shown to act by the removal of an inhibitor, GA does not replace the vernalisation requirement.

There is some supporting evidence that Brian and Hemming's proposal for the effects of GA on internode length may be of fairly widespread application. In fruit trees

(peach and apple), cold treatments are necessary for normal length growth of seedlings and dormant buds. Flemion (1956) suggested that the effect of cold (which is quantitative) is to remove a length inhibitor. Barton (1955) and Denoia and Walker (1957) have found that GA can replace the cold requirement in both peach and crab apple seedlings and dormant buds. Another piece of evidence supporting the idea of a common growth scheme is the work of Parker et al. (1949) who found that the action spectra for stem and leaf growth in etiolated peas was similar to that for the photoperiodically sensitive plants, both long and short day.

Nothing has yet been found out regarding the nature of the inhibitor that GA is thought to neutralise. Brian and Hemming (1957a) have shown that maleic hydrazide is not the natural inhibitor, nor does it simulate it. Many growth inhibitors have been discovered e.g. from Xanthium leaves (Bondo and Khudairi 1954) and from Penicillium (Curtis 1957). Whether these have any connection with the mechanism of GA action is not known.

The effects of GA on cell division and elongation are interesting. In some plants e.g. peas, GA causes mostly cell elongation. In others e.g. Bryophyllum it causes a marked increase in cell division. When it affects elongation, it usually does so in the longitudinal plane. This would be interesting to compare with the effect of benzimidazole, which has been shown to increase the transverse growth of cells (Galston et al. 1953). Sachs and Lang (1957a and b) found that in Bryophyllum, GA promotes cell division in the sub-apical but not the apical region. In addition they found that in normal plants GA induced division was confined to the long axis, whereas when leaf primordia were removed division also took place in the transverse axis. They suggest two possible explanations for this effect: (a) that the leaves place a mechanical restriction on the GA effect, or (b) that when leaves are removed there are additional points of entry into the apex (for GA). The data seem to imply that the effect of GA in increasing cell division in the long axis but not (normally) in the transverse axis may be accidental.

The close connection between auxin and GA metabolism is definitely established, but the nature of the connection is not completely known. Brian and Hemming (1955b) have compared the similarities and differences between the effects of the two substances, as has Kato (1958). In some cases the two substances seem to have opposing effects, for example Zimmerman (1943) simulated virus stunting of leaves with auxin, and Khrancrech (1957) reversed some virus stunt symptoms with GA. In contrast to Brian and Hemming's (1958) findings, some workers have suggested that GA acts as an auxin "sparer" (Vlitos and Meudt 1957a). It may do this by inhibiting auxin oxidase activity (Pilet 1957) although Brian and Hemming found no such inhibition. Netien (1957) working with tissue cultures (parenchyma) obtained a growth inhibition with IAA + GA but no effect with GA alone. This report is of especial interest in view of the recent paper of Osborne (1958) who postulated that one of the effects of IAA on pea internodes may be to combine with some other substance to form a growth inhibitor. The inhibitor suggested by Brian and Hemming (1958) occurs in the same regions of the plant as IAA, and thus may be connected with Osborne's inhibitor.

The different effects of GA on different plants may reflect differences in the competence of tissues to respond to GA. Overbeek et al. (1957) while investigating assay methods, found that leaves and coleoptiles of Avena respond differently to GA, auxins and mixtures of the two. The upper part of the coleoptile responds to auxin and not GA, whereas the leaf enclosed in the lower section of the coleoptile responds to GA but not auxin. In addition, GA enhanced the effect of auxin on the upper part of the coleoptile (thus agreeing with Brian and Hemming) whereas auxin inhibits the GA response in the leaf. An understanding of the reasons for such variation in response could well lead to a better general picture of the interaction between auxin and GA in plants.

Many external similarities exist between the effects of GA on plants and those of kinetin and far-red light. Brian and Hemming (1957b) could find no functional correlation between the effects of kinetin and GA. Vasil (1957a and b) has shown that both substances are necessary for the development of anthers of Allium cepa cultured

in vitro. In a small experiment performed in this department (Sprent unpub.), no effects of kinetin + GA were observed.. However, Vasil's experiment was probably more sensitive in detecting such interactions.

There is no doubt that a relationship exists between the effects of light and the action of GA. Vlitos and Meudt (1957b) found that GA could reverse the effect of red, blue and green light on elongation in Alaska peas. Downs et al. (1957) could control the elongation of beans according to the radiation given. They suggest that the reactions that are involved are the same as those governing photoperiodic and germination effects, thus supporting Evian and Fleming's idea of a common growth scheme. If far-red light and GA both act by the removal of a growth inhibitor, then under the conditions used by Downs et al., GA could not have been completely effective, since GA + far-red light resulted in greater elongation than GA alone.

From the evidence available at the moment, it is difficult to obtain a clear overall picture of plant growth. Most of the major factors involved are probably now known. The chief remaining difficulty is to find out the reasons for the difference in the responses of plants to similar environmental situations.

S U M M A R Y

The presence of a flower inhibitor (colysanthin) as proposed by Barber and Paton for late (Sn) varieties of peas has been confirmed. Colysanthin passes into the plumule during the first two weeks after germination, during which time it can be removed from cuttings by leaching in water. Some evidence has been given to support the hypothesis that a flower promoting substance may be present in the cotyledons of early (sn) peas.

The necessity of light for the destruction of colysanthin does not infer that light is always necessary for flower initiation. Plants which have no colysanthin can initiate flowers in the dark. Light is necessary for flower development in all varieties.

Gibberellic acid modifies many of the morphological and physiological features of growth in peas. Its main effects are:

1. The delaying of flower initiation. This is seldom greater than 2 nodes (regardless of GA dose) and may be connected with the maximum effect of GA on elongation.
2. The inhibition of the production of mature flowers. Flower primordia pass through a time of maximum sensitivity to the action of GA. The number of abortive flowers produced is proportional to the GA dose.
3. The stimulation of internode growth, especially with respect to cell elongation. This is most easily seen in dwarf (lg) varieties. It can also be detected in tall (Le) varieties over the first few internodes. The evidence obtained suggests that the Lg locus may exert its effect on both growth in length and on flowering through the action of some substance with properties similar to the gibberellins, but less strong. If tallness in peas results from the production of a natural gibberellin, then this is not stored in the seed, but can be formed in the early stages after germination.

4. To confirm that the recessive gy_2^c gene is intermediate between Gy_2 and gy_2^s in its action.
5. The delaying of the production of leaves with more than two leaflets.
6. The reduction of leaf area. This response varies with the nutritional environment of the plant.

No conclusive interactionsbetween the effects of GA, temperature and photoperiod on flowering were observed. It is suggested that the effect of GA on flowering in peas may largely result from disorganisation of the apex.

The presence of a leaf "lobing" substance which tends to increase the number of leaflets per node is proposed. This substance is present in the leaves of all varieties and is produced in them under the influence of light. The concentration of "lobing" substance present in the apex can be varied by altering the rate of node formation. This directly effects the nodes at which leaves with more than two leaflets are produced. The "lobing" substance is independent of phyllocaline and caulocaline.

Defoliation treatments and those using chemical substances other than GA were found to have only slight effects upon the growth of peas.

It is suggested that Brian and Hemming's three factor system governing stem growth in peas can also be extended to cover other aspects of vegetative growth. At the moment, insufficient data is available to decide whether or not this system can be extended to reproductive growth.

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